ON THE DEVELOPMENT OF THE STRIO-AMYGDALOID COMPLEX IN THE CHINESE HAMSTER, CRICETULUS GRISEUS

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PROEFSCHRIFT
TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
IN DE GENEESKUNDE
AAN DE KATHOLIEKE UNIVERSITEIT TE NIJMEGEN,
OP GEZAG VAN DE RECTOR MAGNIFICUS PROF. DR. A.J.H. VENDRIK,
VOLGENS BESLUIT VAN HET COLLEGE VAN DECANEN
IN HET OPENBAAR TE VERDEDIGEN
OP VRIJDAG 18 JUNI 1976,
DES NAMIDDAGS TE 2 UUR PRECIES

DOOR
GODEFRIEDUS JOHANNES LAMMERS
GEBOREN TE HENGELO (O)

1976
Krips Repro Meppel
Drukkerij-Uitgeverij Brakkenstein te Nijmegen

Dit proefschrift werd bewerkt in het laboratorium voor anatomie en embryologie (Hoofd: Prof. Dr. H.J. Lammers). Aan allen die aan de totstandkoming ervan hebben meegewerkt, ben ik veel dank verschuldigd. Ik denk met name aan:

- de heren J. Peters, J. v. Wezel en G. Grutters van het dierenlaboratorium voor de verzorging van de Chinese hamsters;
- mevr. I. Vos-Artz, mevr. C. de Vocht-Poort en mevr. I. v.d. Bos-Willemse, alsmede de heer L. Geijsberts voor hun zorgzame bewerking van het histologische materiaal en de vervaardiging van de drie-dimensionale reconstructies;
- de heren J. de Bekker en H. Konings en mej. A. Berends die de fraaie illustraties verzorgden in vruchtbare samenwerking met de heer Chr. v. Huijzen. Deze laatste is ook verantwoordelijk voor de lay-out;
- de heren A. Reijnen en M. Willems voor de vervaardiging van de microfoto's;
- de dames mevr. G. van Son-Verstraeten, mej. A. v.d. Wagen, mevr. J. v.d. Straaten-Podgorski, mevr. M. Peters-v.d. Ing, mej. E. Nas, mej. M. Gerrits voor hun inzet bij de totstandkoming van het manuscript, en mevr. M. Hammond-Westers voor haar adviezen met betrekking tot de Engelse tekst;
- de heer E. de Graaff en zijn medewerkers, de heren A. Wolf en J. Manders, voor hun nimmer in enthousiasme afnemende hulp bij het opsporen van de literatuur;
- mijn vrouw Nelleke, dochter Carolien en zoons Daan en Teun, die mij dikwijls steunden, juist op de momenten dat ik het zo hard nodig had.

Ik denk ook aan hen die indirekt hun stempel drukten op dit proefschrift: mijn leermeesters. In het bijzonder denk ik hierbij aan mijn gesprekken met wijlen Prof. Dr. F.J.J. Buytendijk.

Deze publikatie was mogelijk door ondermeer bijdragen van de Stichting "De Drie Lichten" en de "Ludgardine Bouwmanstichting".

Nu zovele onderzoekers hun werk liever beginnen met het onderzoek van de functie van een organisme, missen zij vaak de vreugde van het ontdekken van de vorm ervan.

naar: E.W. Sinnott

Aan de nagedachtenis van mijn vader

CONTENTS

AAA	area amygdaloidea anterior
AB	nucleus basalis amygdalae
ABL	basolateral amygdaloid complex
ACe	nucleus centralis amygdalae
ACo	nucleus corticalis amygdalae
AL	nucleus lateralis amygdalae
AM	nucleus medialis amygdalae
av	angulus ventralis
bo	bulbus olfactorius
CA	commissura anterior
CC	corpus callosum
CE	capsula externa
CI	capsula interna
cinf	cornu inferius ventric. lateralis
CM	corpus mamillare
CO	chiasma opticum
coar	coarctatio ventriculi
CPC	caudatus - putamen complex
cur	curved plane
di	diencephalon
em	eminence
emth	eminentia thalami
ep	epiphysis
fc	flexura cranialis
fih	fossa interhemisphaerica
fM	foramen Monroi
GP	globus pallidus
he	hemisphere of telencephalon
hes	"Hemisphaerenstiel"
Hi	hippocampus
hyp	hypothalamus
inf	infundibulum
lt	lamina terminalis
lvr	lateral ventricular ridge

mes	mesencephalon
MIC	massa intercalata
mim	massa intermedia
mvr	medial ventricular ridge
neu	neurohypophysis
NO	nervus opticus
NTOL	nucleus tractus olfactorii lateralis
oc	optic cone
os	optic stalk
pal	pallium
plch	plexus chorioideus
rgpr	regio preoptica
rim	recessus inframamillaris
ro	recessus opticus
rpi	recessus pinealis
rpo	recessus postopticus
rpr	recessus preopticus
rsm	recessus supramamillaris
sb	stem bundle
se	septum
SEL	subependymal layer
she	sulcus hemisphaericus
sher	sulcus hemisphaericus ridge (torus hemisphaericus)
sia	sulcus intra-encephalicus anterior
SML	submaternal layer
ssd	sulcus subpallii dorsalis
ssi	sulcus subpallii intermedius
ST	stria terminalis
st	sulcus terminalis
sub	subthalamus
tel	telencephalon
thal	thalamus
TO	tractus opticus
tup	tuberculum posterius
ttr	torus transversus
ve	ventricular elevation

vetr	velum transversum
vi	ventriculus impar
v III	ventriculus tertius
vl	ventriculus lateralis

I INTRODUCTION

The subdivision of the brain into fundamental morphological entities has been a matter of conflict for many years. On the basis of neuroembryological studies His (1893a) concluded that the brain stem can be subdivided into two longitudinal zones: the ventral, primarily motor, basal plate and the dorsal, primarily sensory, alar plate. According to His, the boundary between these zones is demarcated on the ventricular side by a groove: the sulcus limitans. A subdivision of the diencephalon into four longitudinal zones was suggested by Herrick ('10) as a result of his studies on the amphibian brain. Evidence for the existence of a longitudinal zonal pattern in the vertebrate telencephalon and diencephalon was provided by Kuhlenbeck ('29a, '30). In these papers Kuhlenbeck expressed the opinion that in all vertebrates the brain parts mentioned have a basic pattern or "Bauplan" in common. In this concept great value was attributed to the ventricular sulci, forming the boundaries between the longitudinal zones.

Quite a different subdivision of the brain was suggested by a number of authors who studied the ontogenesis of the vertebrate central nervous system. These investigators (among them Von Kupffer, '06; Bergquist, '52; Bergquist and Källén, '54, and Vaage, '69) arrived at the conclusion that the transversally orientated neuromeric bulges, which originate during early ontogenesis, represent the basic morphological elements of the vertebrate brain. When the neuromeres disappear and are replaced by "migration areas", secondary evaginations develop which give rise to the well-known five brain vesicles (Bergquist and Källén, '54). Contrary to the neuromeres these vesicles are not transitory structures but remain distinguishable during further development up to the adult stage. Different opinions however, exist in literature about the number of neuromeres present, as reviewed by Keyser ('72). However, all authors mentioned unanimously stated that no neuromeric bulges could be recognized within the telencephalic hemisphere. Morphologically the lateral telencephalic wall is usually subdivided into a dorsal and a basal part, which in mammals during further development give rise to the pallium and subpallium, respectively.

From this thematic survey it can be concluded that different opinions exist in literature concerning the fundamental morphological pattern of the brain. It should, however, be emphasized that the morphogenesis can hardly be studied without an analysis of the underlying histogenetic events.

The present day concept of the histogenesis is based upon a subdivision of the wall of the neural tube into three layers: the inner matrix layer (or ventricular zone), the mantle layer (or intermediate zone) and the outer marginal layer (or marginal zone). During ontogenesis in principle three histogenetic processes can be discerned: (1) proliferation or cell production; (2) migration or displacement of the cells from their site of origin to their ultimate position and (3) differentiation, in which the migrated cells differentiate into nerve or glial cells and establish their functional connections. The proliferation occurs within the matrix layer, in which all of the constituent cells actively take part in the cell production and during their mitotic cycle perform a so-called elevator movement to and from the ventricular surface, as was autoradiographically established by Fujita ('62). From a certain moment onward, the cells leave the matrix layer to enter the mantle layer and migrate towards their final destination. The neuroblasts, having lost the capacity of mitotic division, group themselves and differentiate into mature neurons, forming the ultimate grisea.

At least one exception to the three-layer concept alluded to above has to be mentioned, namely the telencephalic subpallial area. In this region a fourth layer develops in between the matrix and mantle layer, the so called subependymal layer or subventricular zone. Its constituent cells are characterized by their proliferative activity, but in contradistinction to the matrix cells they do not exhibit an elevator movement to and from the ventricular surface. The histogenetic processes described above do not take place at the same time in the different parts of the central nervous system. This phenomenon is called heterochrony. His ('04) and Streeter ('11) already noted that basal parts develop earlier than dorsal parts, whereas Spatz ('27) observed that rostral parts lag behind as compared with caudal parts. Both the basodorsal and the caudorostral gradient were more recently substantiated by the findings of Kahle ('51, '56, '58) in his analysis of the development of the matrix layer in human material. The heterochrony is also expressed by the time of origin of the neurons constituting the various brain nuclei, as can be established by the autoradiographic technique. The heterochrony of both the development of the matrix layer and the appearance of the various brain nuclei support the idea that the neurons contributing to a particular brain nucleus are generated within one and the same matrix area, as was also suggested by Morest ('70).

The morphological and histological concepts as described above form the background of the present investigation of the development of the strio-amygdaloid complex. In the adult Chinese hamster this complex is mainly situated in the basal part of the telencephalon. The striatum is composed of the globus pallidus and the caudatus-putamen complex. It is stressed here that the nucleus accumbens is not taken into consideration, although some authors regard it as a component of the striatum (cf. Swanson and Cowan, '75). Within the corpus amygdaloideum a basolateral group of nuclei (nucleus basalis and nucleus lateralis), a corticomedial group (nucleus corticalis, nucleus medialis and nucleus centralis) and a rest group (nucleus tractus olfactorius lateralis, area amygdaloidea anterior and massa intercalata) can be distinguished.

As regards the ontogenesis of the strio-amygdaloid complex, most authors consider the entire complex as a subpallial derivative. Spatz ('24, '25), however, suggested that the globus pallidus originates from the diencephalon. Källén ('51a) held that the nucleus basalis and the nucleus lateralis amygdalae are of pallial origin. However, in order to determine the site of origin of the various strio-amygdaloid nuclei the development of the matrix layer of the area in question has to be analysed. Secondly, a thorough knowledge of the morphogenesis is indispensable. It has been known for a long time that during a certain developmental period two ventricular ridges are present within the basal part of the telencephalic hemisphere. Some authors (Johnston, '23; Kodama, '27, and Kahle, '69) supposed that the neurons constituting the caudatus-putamen complex are generated within the matrix of only one of these ridges, whereas Hochstetter ('19) suggested that these neurons would be derivatives of both ridges. Similarly, divergent opinions exist in the literature concerning the site of origin of the neurons constituting the amygdaloid complex (cf. Hewitt, '58; Brown, '67, and Humphrey, '68; '72).

This thematic survey shows that regarding the ontogenesis of the strio-amygdaloid complex not only a number of controversies exist in literature but also many aspects of the development of this complex are still to be elucidated. The aim of the present investigation is to study the development of the strio-amygdaloid complex in a mammal, the Chinese hamster. It will be attempted to answer the following questions:

1. In which way do the ventricular ridges mentioned above develop, in relation to their surrounding areas and what is their final fate?
2. What are the developmental characteristics of the matrix layer in the area under consideration?

3. When do the various grisea constituting the strio-amygdaloid complex develop and what are their topographical relationships?
4. At which time of development are the neurons contributing to these grisea generated?
5. Is it possible to determine the site of origin of the neurons of each griseum within the matrix layer?

In the first part of the present investigation a survey is given of the material, techniques and methods used in this study. Besides, the various developmental stages are characterized (Chapter II). The early morphogenesis of the mammalian central nervous system is reviewed in chapter III, in which a general terminological background is also provided for the considerations to be presented in the following chapters. Secondly, in this chapter some landmarks are introduced, which are indispensable for an adequate description of the morphogenesis. The latter forms subject of chapter IV, in which special attention is paid to the morphological changes of the ventricular surface. In order to facilitate this description both graphical and three-dimensional reconstructions have been made.

In chapter V the development of the neuroepithelium and in particular that of the matrix layer is investigated. This study was carried out by subdividing the process into different subsequent phases. The functional state of the matrix layer was analysed accordingly and the matrix regions, each of which exhibiting one particular phase, were graphically reconstructed. By comparing the maps obtained at the various developmental stages, the heterochronous development of the different areas could be established. The differentiation of the grisea constituting the strio-amygdaloid complex and the development of some main fibre bundles is studied in chapter VI. The time of appearance of the grisea was recorded. Secondly, the changing topographical relationships of the various structures is described with the help of graphical reconstructions. This part of the histogenesis is supplemented by an autoradiographic investigation of the time of origin of the neurons constituting the various grisea of the strio-amygdaloid complex (Chapter VII).

In chapter VIII an attempt is made to give an integral survey of the results obtained. First, some morphogenetic events are discussed in view of the underlying histogenetic processes. Secondly, for each nucleus the time of origin of its constituent neurons will be compared with its first histological

recognizability. In this way some information concerning the migratory paths of the neurons will be obtained. Finally, the functional state of the matrix layer at the respective times of origin of the neurons of each particular nucleus may give an idea of the site of origin of the elements concerned.

2 MATERIAL, TECHNIQUES AND METHOD

2.1 *The Chinese hamster*

The material employed in the present study consists of embryonic, postnatal and adult brains of the Chinese hamster (Cricetulus griseus). The Chinese hamster is a very useful animal for ontogenetic studies because of several reasons.

1. The gestation period of the Chinese hamster is rather short, it lasts approximately 21 days.
2. The fertility of the Chinese hamster is quite favourable: an average of 4 to 6 littermates is usual.
3. The breeding method as described by van Gaalen ('63) enables a rather accurate determination of the postconceptional age of Chinese hamster embryos. The inexactitude amounts to plus or minus one and a half hours only.
4. In using the Chinese hamster we could take advantage of the experience and the results obtained at our laboratory in a previous investigation (Keyser, '72).

2.2 *The developmental stages*

Every ontogenetic study is based upon a comparison of different developmental stages. This implies that each developmental stage has to be accurately defined in order to be able to decide to which developmental stage the individual embryos belong. Several authors emphasized that other criteria than the postcoital age have to be used in staging the embryos (O'Rahilly and Gardner, '71, O'Rahilly, '72; Javor, '74; Gribnau and Lammers, '76). This is the more necessary in the Chinese hamster, since variations in development even occur between littermates, as was also described for other rodentia by Ziehen ('06).

Apart from the postconceptional age, the staging of the embryos in our study is performed according to three sets of criteria (cf. Gribnau and Lammers, '76): (1) data derived from the whole embryo, such as outer size and/or shape; development of outer structures; (2) the macrostructure of the central nervous system, i.e. size and/or shape; development of macroscopically recognizable structures. (3) the microstructure of the central nervous system, in which both morphological and histological aspects are involved.

In the older developmental stages (16 and 18 days post-conception) the characteristics mentioned under 2 are directly available after dissection of the brains out of the skull. In the younger stages the information needed can be obtained after the embryos have become cleared in methylbenzoate.
The material employed in the present study consists of embryos ranging from embryonic day 12 (E 12) to embryonic day 18 (E 18), and of brains of postnatal animals 3 days of age (PN3) and 100 days of age (adult).
The developmental stages of the embryos analysed are characterized as follows:

The stage E 12 + 7 hours

- Upper limb bud present
- Lower limb bud absent
- Formation of optic cup is initiated
- Otic vesicle closed
- Flexura cranialis dominates morphology of the CNS
- Initial flexura cervicalis present
- Evagination of cerebral hemispheres has started
- Plica encephali dorsalis present
- Formation of the infundibulum is initiated

The stage E 12.5

- Hind limb bud distinguishable
- Flexura cervicalis distinct
- Initial flexura pontina present
- Cerebral hemispheres present
- Future position of epiphysis recognizable
- Medial ventricular ridge present

The stage E 13

- Handplate present
- Hind limb bud distinct
- Retinal pigment present
- Slight pontine flexure present
- Primordial epiphysis recognizable
- Commissura posterior fibres present

The stage E 13.5

- Footplate present
- Formation of external ear is initiated
- Beginning closure of the optic stalk
- Lateral ventricular ridge present
- First differentiation of fibre bundles in the prosencephalon (stem bundle, stria medullaris)

The stage E 14

- Within the handplate fingerrays appear
- Optic stalk closed
- Bulbus olfactorius region present
- First fibres of the tractus opticus present

The stage E 16

- Within the footplate toerays present
- Externally distinguishable elbow
- The cerebral hemispheres cover the main part of the diencephalon
- Plexus choroideus present in the lateral ventricles
- Formation of the plexus choroideus in the third ventricle roof initiated
- First fibres of the commissura anterior present

The stage E 18

- Pronounced outgrowth of the splanchnocranium as compared with the preceding stage
- Most of the diencephalon is covered by the cerebral hemispheres
- In the diencephalon all primordial mantle layer structures are present
- First corpus callosum fibres present

2.3 *Normal material*

Embryonic material of varying developmental ages was collected after sacrificing pregnant hamsters under ether anaesthesia. The embryos were removed immediately from the uterus and fixed by immersion in either Bouin's or Carnoy's fluid (Romeis, '68). In order to obtain well fixed brains of animals 3 days postnatal of age, the specimens were decapitated under ether anaesthesia. After opening of the skulls the heads were fixed by immersion in

either of the two fluids mentioned for 12 hours. Subsequently, the brains were removed from the skulls and replaced in the respective fixation fluid for at least 24 hours. Adult Chinese hamsters were perfused under deep nembutal anaesthesia with Bouin's or Carnoy's fluid. Additionally, the brains were fixed by immersion in either solution for 24 hours.

After fixation the specimens were processed in alcohol (progressively ranging from 50 to 100%), methylbenzoate, amylacetate and paraffin-wax according to a time schedule which is adapted to the size of the material (Romeis, '68).

In view of the preparation of the reconstructions the lateral aspects of the CNS of the specimens are photographically recorded either directly after fixation (PN_3 and adult stage) or during the methylbenzoate phase (embryonic material). Since the direction of sectioning is of paramount importance for the preparation of the reconstructions the material has to be well orientated during embedding in paraffin-wax. For detailed information on this subject the reader is referred to Gribnau and Lammers ('76).

The material was serially sectioned in one of the three conventional directions at 7 or 10 micra and stained according to Mayer's modification of the haematoxyline and eosine technique or a modification of the Nissl technique (Romeis, '68). For the study of the developing fibre bundles the sections were impregnated according to Bodian ('36) or Palmgren ('48), whereas in the adult stage the staining according to Klüver-Barrera ('53) was also used. A survey of the material employed is presented in table 1.

	Number of series				
Age of the specimen	HE staining	Nissl staining	Bodian staining	Palmgren staining	Kl. Barrera staining
E 12d + 7h	6	1		1	
E 12.5d	5				
E 13d	20		5	1	
E 13.5d	15		1	3	
E 14d	31		12	15	
E 15d	20	3	7	8	
E 16d	20	4	7	8	
E 18d	25	3	4	4	
PN 3d	7	3	4		3
PN 100d	10	10	4	2	10

Table 1. Survey of the normal material employed.

2.4 *Autoradiographic material*

The material employed in the autoradiographic part of this study consists of adult brains of animals which had received a single dose of tritiated thymidine at varying developmental ages. The administration of the thymidine was accomplished either via an intra-uterine injection or by way of an injection into the peritoneal cavity of the mother. In case of an intra-uterine injection each embryo received a fixed dose of tritiated thymidine (spec. act. 5 Ci/mmol) adapted to its age according to Berry and Rogers ('65) as follows: E_{12} receiving 10 μCi/embryo; E_{13} and E_{14} 15 μCi/embryo; E_{15} and E_{16} 20 μCi/embryo and E_{17} 25 μCi/embryo. The intra-peritoneal dose was calculated as 5 μCi per gram maternal bodyweight as adopted from Angevine ('65, '69) and Altman ('66).

The material was fixed in Carnoy's solution, dehydrated and embedded in paraffin-wax. It was then serially sectioned at 7 micra, mostly in the transverse direction and mounted on slides. The slides were coated in the dark with radiosensitive Ilford G5 or K5 emulsion according to the dipping technique as described by Berry and Rogers ('65). The slides were kept at 4°C in a lead box for protection against environmental radiation. The exposure time amounted to approximately four weeks. Subsequently, the sections were developed with an amidol developer at 15°C during 10 minutes and fixed with 30% sodium thiosulfate. The sections were stained with either haematoxyline and eosine or toluidine blue. A survey of the autoradiographic material employed is presented in table 2.

With regard to the interpretation of the autoradiographs, it should be noted that those cells showing more than 15 grains per nucleus were defined, although arbitrarily, as heavily labeled cells.

Survival time of the specimen after injection with thymidine H^3	Number of series
$E_{12} \rightarrow$ adult	5
$E_{13} \rightarrow$ adult	5
$E_{14} \rightarrow$ adult	4
$E_{15} \rightarrow$ adult	6
$E_{16} \rightarrow$ adult	5
$E_{17} \rightarrow$ adult	4

Table 2. Survey of the autoradiographic material employed.

2.5 *The reconstruction technique*

The present study is to a very large extent based upon observations derived from graphical and three-dimensional reconstructions. In the reconstruction techniques used the presence of a longitudinally oriented plane of symmetry in the (developing) central nervous system was postulated. The method is based upon the combination of data derived from two different series of sections of each developmental stage. These two series are sectioned both at right angles to the median plane and perpendicular to each other, namely in the horizontal and transverse direction respectively. The reconstruction technique comprises first the graphical reconstructioning of the median sections of both specimens and, secondly, a comparison and optimalization of these graphical reconstructions. Since a detailed description of the reconstruction technique is published elsewhere (Gribnau and Lammers, '76) only the subsequent steps of the procedure will be summarized here, as follows:

1. drawing of the sections of both series; the final product of each section is a straight line, representing the median line, which is carrying two different sets of points, namely a) the points of intersection with median structures and b) the projections of the points of greatest extension of the outline in a direction parallel to the median line;
2. drawing of the contour tracings of the central nervous system of both specimens, using the photographically recorded lateral aspects obtained as described previously. In the contour tracings a system of equidistant lines is introduced in the direction of sectioning of the series in question. The distance between the lines is calculated from the section thickness the distance between two consecutively analysed sections and the magnification;
3. graphical reconstructioning of the median sections of both specimens in the following way: the resulting straight lines (sub 1) are transferred in sequence on the equidistant lines (sub 2), in which the outline tracing serves as an initial guide for the alignment of both sets of points. The reconstructions of both median sections are completed on the basis of the best fitting curves;
4. comparison and optimalization of the two graphically reconstructed median sections. Corrections required are introduced by shifting the lines together with both sets of points as derived from the sections (thus without changing any distance between the points) along the corresponding

equidistant lines.

This procedure results in two optimalized reconstructions of the median sections of the central nervous system of both specimens. Subsequently, each of these reconstructions is employed as a reliable basis in the preparation of graphical or three-dimensional reconstructions of the central nervous system in question. The graphical reconstructions are made using the orthogonal projection technique as reviewed by Gaunt ('71). The three-dimensional reconstructions are prepared using polystyrene plates, as described by Gribnau and Lammers ('76). The reconstructions employed in the present study are summarized in table 3.

Age of the specimen	Three-dimensional reconstruction	Graphical reconstruction
12d + 7h	+	+ (2x)
12.5d	+	+ (2x)
13d	+	+ (2x)
13.5d	+	+ (2x)
14d	+	+ (3x)
16d	+	+ (3x)
18d	+	+ (4x)
PN 3d		+ (3x)
PN 100d		+ (3x)

Table 3. Survey of the reconstructions employed.

2.6 *Method*

In the developmental period covered by the present investigation the central nervous system is transformed from a simple neural tube into the highly complex adult brain. Although these transformations are reflected in rather drastic changes of the morphology of the central nervous system at various developmental stages, the preservation of the continuity of both the inner and outer surface of the brain wall is of fundamental importance in an analysis of the morphogenesis.

The morphological transformations can be described as isolated events, in our opinion, however, the morphogenesis should be interpreted as resulting

from the histogenetic processes occurring within the wall of the central nervous system, especially during early ontogenesis. On the other hand the histogenetic events can hardly be studied without a thorough knowledge of the morphogenesis, since otherwise misinterpretations of the sectioned material would be inevitable. On that account the first analytical chapter of the present study will deal with the morphogenesis, whereas the various aspects of the histogenesis will be the object of the following chapters. Finally, an attempt will be made to integrate these two main issues of the ontogenesis of the central nervous system.

3 MORPHOLOGICAL AND TERMINOLOGICAL INTRODUCTION

3.1 *Introduction*

The aim of the present investigation is to give a description of the morphogenetic and histogenetic events which occur during the development of the Chinese hamster prosencephalon after the closure of the anterior neuropore. However, for a better understanding the literature concerning the early morphogenesis of the mammalian central nervous system will be briefly reviewed in the first part of this introductory chapter. In the second part a general terminological background will be provided for the description and considerations presented in the later chapters. Finally, some midline structures which serve as landmarks in the prosencephalon will be discussed.

3.2 *The early development of the mammalian central nervous system*

3.2.1 *The formation of the neural tube*

In late presomite stages the embryonic disc consists of three fundamental germ layers: ectoderm, mesoderm and entoderm. Soon after the formation of the primitive streak caudally, which extends rostrally to Hensen's node, in front of the latter structure an axial thickening of the ectoderm is established. This ectodermal formation is called the neural plate.

In 1903 Spemann suggested that the formation of the neural plate might be causally influenced by the underlying mesodermal archenteron (= primitive gut). This hypothesis was experimentally affirmed by several authors (Spemann; '18; Spemann and Mangold, '24; Lehman, '29 and others).

Many subsequent investigations about the nature of the inductive system have been carried out as reviewed by Spemann ('36, '62), Saxén and Toivonen ('62) and more recently by Nieuwkoop ('73). However, the nature of the induction mechanism is still not entirely elucidated, although it is quite probable that chemical processes play an important role (Bautzmann, '32).

In early somite stages the lateral margins of the neural plate, called neural folds, become elevated to form the neural groove. The neural folds continue to raise until they meet each other dorsally in the midline and fuse. In this way the neural plate is transformed into the neural tube, which process is called neurulation. Subsequently, the neural tube becomes detached from the ectoderm and sinks as a whole beneath the latter.
Some authors believed the neurulation process to be resultant from mechanical

forces, originating either externally in mesodermal parts of the embryo (His, 1874; Jacobson, '62) or internally within the neural plate itself (Weiss, '55). Other authors, among them Jelinek and Friebova ('66) and Langman et al. ('66) supposed that the formation of the neural tube might be caused by different proliferative activities within the wall of the neural groove. However, the problem of the mechanism underlying the neurulation process remains to be solved.

It has been known for a long time that the transformation of the neural groove into the neural tube does not occur simultaneously along the whole groove (His, 1868; Von Mihalkovicz, 1877; Von Kupffer, '06). The fusion of the neural folds probably commences at the presumptive cervical region and proceeds from there rostrally and caudally. Finally, also the ultimate openings at either end of the tube, called the anterior and posterior neuropore respectively, become closed.

As regards the anterior neuropore no unanimity exists in literature concerning the mode and the exact location of its closure. His (1893) and Bartelmez and Dekaban ('62) held that a fusion of the neural folds starting at the presumptive optic chiasm and proceeding dorsally would occur in addition to the rostrally directed fusion initiated at the cervical level. According to these authors the last point of closure would be located at the site where both processes meet each other.

Great stress was laid on the position of the anterior neuropore since it has been frequently used as indicating the rostral end of the central brain axis (Grönberg, '01; Johnston, '09, '13, among others). Some authors supposed that the last point of closure may vary in position (Davies, '23; Streeter, '42; Bartelmez and Evans, '26; Bartelmez and Dekaban, '62). However, the difficult traceability of the anterior neuropore after its closure has contributed a great deal to the dispute in literature about its position (cf Tandler and Kantor, '07; Johnston, '09; Hines, '22), although Von Kupffer ('06) and Jonhston ('09) reported a recessus neuroporicus to be left as a remnant in most vertebrates. In the Chinese hamster Keyser ('72) could not provide evidence for the existence of such a recessus. Summarizing the literature it can be noted that the rostral end of the central brain axis generally is defined somewhere between the torus transversus and the velum transversum.

3.2.2 *The fundamental parts of the neural tube*

Already before the closure of the anterior neuropore the anlage of the central nervous system can be subdivided into a narrow elongated caudal part and a wider rostral part, which were termed myelon and encephalon respectively (Mc Clure 1890). The encephalon will become the brain area. Within this area Von Kupffer ('06) discerned two vesicle-like structures which he termed the archencephalon and the deuterencephalon; the latter caudally grading into the myelon. Soon after the appearance of the two vesicles just mentioned, the deuterencephalon is subdivided into two parts: the mesencephalon and the rhombencephalon. Now the brain area of the neural tube consists of three vesicles: the archencephalon, which from now on is termed prosencephalon, the mesencephalon and the rhombencephalon. Further development of the primordial brain occurs in such a fashion that the prosencephalon and the rhombencephalon both give rise to two secondary vesicles. The five constituent brain compartments are named from rostral to caudal telencephalon, diencephalon, mesencephalon, metencephalon and myelencephalon (Huxley, 1871).

As regards the development of the telencephalon, which will be elaborated in the following chapter, it has to be mentioned here, that its lateral walls become subjected to a strong evagination which leads to the formation of the two lateral hemispheres. The lumen of each cerebral hemisphere also termed the lateral ventricle, maintains an open communication with the third ventricle via the interventricular foramen of Monro. However, the most rostral part of the prosencephalic vesicle does not participate in the evagination. This unevaginated part and the lumen belonging to it are termed the telencephalon medium and the ventriculus impar, respectively. Although many previous authors accepted the subdivision of the brain tube into five vesicles (His, 1893a, 1895, '04; Von Kupffer, '06; Ziehen, '06; Johnston, '09, '23) some other authors, among them Streeter ('33); Bergquist ('52); Bergquist and Källén ('54) and Vaage ('69), considered quite different structures, namely the neuromeres, as the fundamental morphological entities of the neuraxis. These authors unanimously considered the neuromeres as primary structures, whereas in their opinion the five vesicle model would only have secondary value. Neuromerism can be described as the phenomenon that in early stages the neural tube is characterized by a longitudinal segmental organization into bulges alternating with constrictions. These bulges were first defined by Orr (1887), who also coined the term neuromeres. Von Kupffer ('06) provided evidence that even before the closure of the anterior neuropore the neural plate already

displays a metameric pattern. He proposed the name primary neuromeres for these early structures as opposed to secondary neuromeres indicating the bulges after closure of the neural tube. Rosenbauer ('55) considered the neuromeric structures as artifacts. However, observations on living chick embryos (Källén, '55; Bergquist, '56; Vaage, '69) convincingly showed that neuromeres do exist. Bergquist ('52), Bergquist and Källén ('53b) and Källén ('52, '56) provided evidence that neuromeric bulges coincide with proliferation maxima. Accordingly, they suggested that centres with a high proliferative activity alternating with parts having a low activity would cause the formation of neuromeric bulges and constrictions, respectively. Some different opinions exist about the number of prosencephalic neuromeres (for a review see Keyser, '72). In the telencephalon no distinct neuromeric structures have been discerned (Bergquist and Källén, '54; Vaage, '69). On that account neuromerism will not be taken into consideration in the present investigation.

3.2.3 *The cerebral flexures*

Already before the subdivision of the deuterencephalon into mesencephalon and rhombencephalon the central axis of the neural tube displays a dorsally convex curvature. This curvature was called flexura cephalica by His (1888), plica encephali ventralis by Von Kupffer ('06) and flexura cranialis by Bartelmez and Evans ('26). Morphologically this flexure is of considerable importance, since it indicates the position of the primordial mesencephalon. Moreover, it persists throughout development and later on becomes even more accentuated. During further development a second, dorsally convex, curvature originates at the junction between rhombencephalon and spinal cord. A third curvature arises at the rhombencephalic level, pari passu with the subdivision of the rhombencephalon into metencephalon and myelencephalon. In contradistinction to the two flexures mentioned above this third one, called the flexura pontina, shows a ventral convexity.

Summarizing it can be noted that the early morphogenesis of the vertebrate central nervous system is characterized by three features:

1) the transformation of the neural plate into the neural tube;
2) the subdivision of the primordial brain into its fundamental parts;
3) the genesis of the three cerebral flexures.

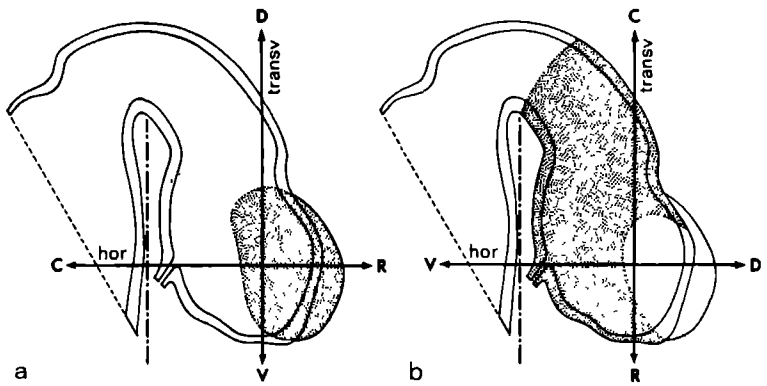

fig. 1 Orientation and terminology employed in the description of the development of the telencephalon (a) and the diencephalon (b).

The continuous transformation of the neural tube during development hampers the orientation within the primordial vertebrate brain. The use of the central brain axis as a reference is very inconvenient because of the cerebral flexures. For that reason in most neuroanatomical studies the topographical positions are described according to the convention in which the central brain axis is thought of as unrolled. The neuroporus anterior, however, which would indicate the rostral end of the brain axis, is hardly traceable after its closure. Keyser ('72) was not able to discern a recessus neuroporicus in the Chinese hamster. Nevertheless, the orientation in the forebrain is very important in view of the terminological difficulties which would arise otherwise. Therefore, we decided to define in each developmental stage the horizontal as well as the transverse plane, which are both perpendicular to the median plane. In prenatal stages the direction of the horizontal plane is defined as being perpendicular to the flexura cranialis (cf. fig. 1), whereas in postnatal stages that direction is determined by a plane through the optic chiasm and the basis of the bulbus olfactorius. Accordingly, the transverse plane is defined as being perpendicular to the horizontal plane. The way in which the terms dorsal, ventral, rostral and caudal will be employed in the description of the development of the telencephalon, is elucidated in figure 1a. It should be emphasized that in the

description of the diencephalon these terms will be used in a different way (fig. 1b).

3.4 *Landmarks*

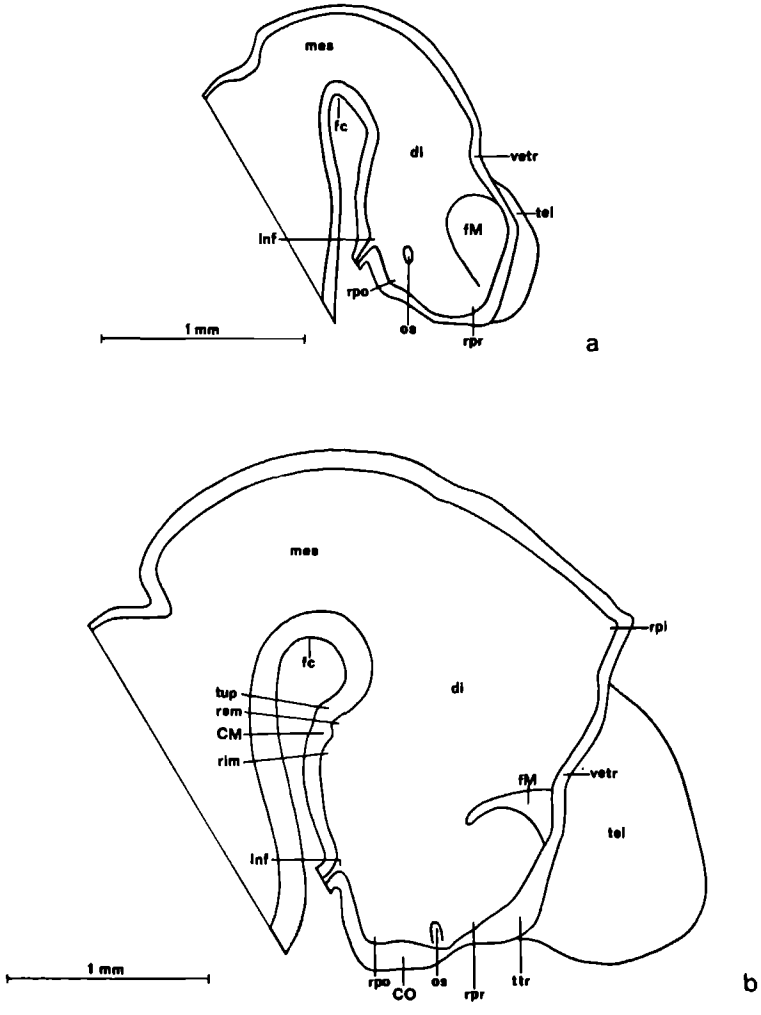

fig. 2 Configuration of the midline structures of the rostral part of the neural tube at the stages E12+7h (a) and E13.5 (b).

It should be appreciated that an investigation of the development of the central nervous system consists of a synthesis of a number of static pictures of different developmental stages, which each form an instantaneous view of a dynamic process. This limitation is inherent to most embryological research but particularly applies to a description of the continuously transforming neural tube. Such a synthesis is only possible when certain invariants are available throughout development.

During the ontogenesis of the central nervous system certain structures situated in the median plane manifest themselves early in development and remain recognizable up to the adult stage. These structures therefore can be used as reference points in the orientation. We will introduce these landmarks with the aid of figure 2.

The first morphological characteristic of the rostral part of the neural tube is the flexura cranialis, which as mentioned previously indicates the position of the mesencephalon on top of it. The diencephalon is located in front of the mesencephalon. The dorsal border between these two brain parts is in later development marked by the commissura posterior. The epiphysis, which in early development is indicated by a small pit: the recessus pinealis, is situated more rostrally in the diencephalic roof.

The dorsal border between the diencephalon and the telencephalon medium in indicated by the velum transversum. Ventrally to this structure a local thickening of the wall can be observed, which will be named torus transversus in the present study as it was called by Von Mihalkovicz (1877). It corresponds with the "concrescentia primitiva" of Grönberg ('01) and the "Kommissurenplatte" of Hochstetter ('19). A number of authors consider this structure as indicating the basal telodiencephalic boundary (Von Mihalkovicz, 1877; Tandler and coworkers, '07, '15; Kuhlenbeck, '54).

In later development the commissura anterior will originate within the torus transversus. Caudal to the latter structure a shallow pit can be observed: the recessus preopticus. We will conform ourselves to many authors, among them Von Mihalkovicz (1877) and Keyser ('72), in defining the lamina terminalis as the stretch of epithelium situated between the velum transversum and the recessus preopticus.

The recessus preopticus is situated in front of a second thickening of the wall: the primordial chiasma opticum, which in its turn is bordered by a second pit: the recessus postopticus. The basal wall of the diencephalon is constituted by the rostral limb of the flexura cranialis. Within the latter

structure rostrally the infundibulum can be discerned, whereas more caudally the presumptive corpus mamillare is situated. The last structure is bounded by two small pits, the recessus inframamillaris and supramamillaris respectively. Finally, just in front of the top of the flexura cranialis, the tuberculum posterius is located.

In the following chapters the observations and considerations concerning the ontogenesis of the central nervous system in the Chinese hamster will be described in reference to the landmarks mentioned.

4 THE MORPHOGENESIS OF THE LATERAL AND BASAL PART OF THE HEMISPHERE WALL

4.1 *Introduction*

Many investigators studying the ontogenesis of the brain, have emphasized the importance of the morphogenesis of the ventricular surface with special regard to the furrow pattern. This pattern has been extensively studied by Herrick ('10, '33, '48), Johnston ('09, '13, '23), Kuhlenbeck ('29a, '33, '36, '37, '70), Bergquist ('32, '52), Bergquist and Källén ('53a, '54) and Källén ('51a, b, c, '55b). These authors have also paid attention to the relation between the ventricular sulci and the developmental events occurring in the wall of the diencephalon. This relation between the morphogenesis and the histogenesis is of great importance, although a number of different opinions exists about its interpretation. For this reason it is necessary to study the morphogenesis of the ventricular surface, which enables the comparison of the data obtained from the literature with those found in our investigation. A second reason to study the morphogenesis especially of the lateral and basal part of the hemisphere wall is, that in most previous investigations a description is given, without illustrations showing a clear spatial picture.

4.2 *Literature*

In the literature a consensus of opinion exists about the way in which the telencephalic vesicles originate in vertebrates. The telencephalic hemispheres arise as lateral evaginations of the thin-walled prosencephalon, which then becomes subdivided into the diencephalon and telencephalon. On the external surface the telodiencephalic boundary can be easily recognized by a distinct groove, called the sulcus hemisphaericus (Grönberg, '01; Hochstetter, '19) or sulcus telodiencephalicus (Tandler and Kantor, '07; Bartelmez and Dekaban, '62). On the ventricular side this sulcus corresponds to a curved ridge: the sulcus hemisphaericus ridge or torus hemisphaericus (Kuhlenbeck, '29), or prominentia telodiencephalica (Tandler et al., '07; '15). The cavity of each telencephalic hemisphere, the lateral ventricle, communicates with the third ventricle through a wide interventricular opening, the primitive foramen of Monro. After their origin the thin-walled telencephalic hemispheres expand dorsally, rostrally and caudally. Basally their walls soon start to thicken. In consequence of this differential growth the walls of the cerebral hemispheres can be subdivided into a thin-walled pallial region and

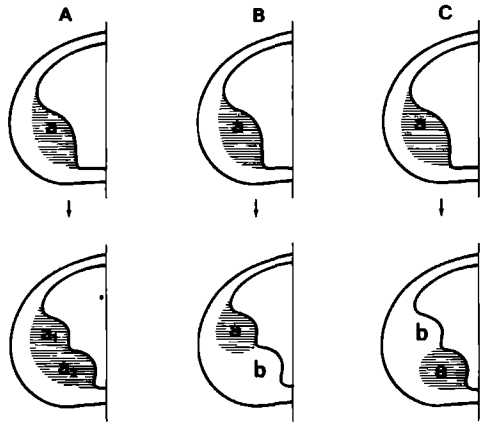

fig. 3 Schematic representation of the origin of the second ventricular ridge, as suggested by Hochstetter ('19), A; Humphrey ('68), B and Hewitt ('58), C.

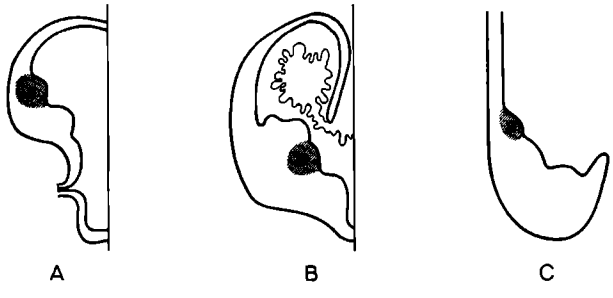

fig. 4 Schematic representation of the position of the third ventricular ridge, as suggested by His ('04), A; Hochstetter ('19), B; Källén ('51a), C. Note the different directions of sectioning!

a thickened subpallial region.

According to most authors the basal, elevated area forms during later development two longitudinally oriented ventricular ridges. It has been known for a long time that within these ridges the corpus striatum (nucleus caudatus and putamen) develops. For that reason often a less adequate nomenclature was used in respect to the ridges, such as "corpus striatum" or "striatal elevation" (de Vries, '10; Hines, '22; Cooper, '46; Hewitt,

'58, '61; Bartelmez and Dekaban, '62; Brown, '67; Humphrey, '68) or "Streifenhügel" (His, 1890, 1892, '04). In our opinion these denotations are very inconvenient, since the corpus striatum must be considered as an adult structure and not as an ontogenetic one, as was already stated in 1816 by Tiedemann. Therefore in the present study the elevated structures will be called ventricular ridges. Concerning the development of the ventricular ridges no unanimity appears to exist in the literature. The first matter of dispute is the localization of the ridges. However, the allied problems can be reduced mainly to the question of the telodiencephalic boundary. In early development this boundary is clearly marked on the inside by the torus hemisphaericus, which rostrally fades towards the torus transversus. Basally this boundary becomes obscured by the development of the first originating ventricular ridge, which is situated in the floor of the interventricular foramen. Although most authors considered both ventricular ridges as telencephalic structures, some others supported the view that the medial one has a mainly diencephalic origin (Spatz, '24, '25; Grünthal, '52). On the other hand Tandler et al. ('07, '15) considered the ventricular ridges as a lateral outgrowth of the median torus transversus.

Secondly, some different opinions exist in the literature concerning the way in which the two ventricular ridges do originate. Most reports are consistent about the fact that during development at first one and later on two ventricular ridges exist. A number of authors supposed that at a certain time of development the first, single ridge becomes subdivided into a medial and a lateral part (Tiedemann, 1816; v. Mihalkovicz, 1877; Hochstetter, '19; Källén, '51a; Hamilton et al., '72). Other authors stated, however, that the two ridges do not arise at the same time of development. Most of them (Kodama, '26; Grünthal, '52; Hewitt, '58, '61; Brown, '67; Kahle, '69, among others) suggested that the medial ventricular ridge arises before the lateral one, whereas Humphrey ('68) supposed that the sequence of their appearance is the reverse. The various opinions are schematically shown in figure 3.

For several groups (primates, rodents, birds) evidence was provided for the existence of two ventricular ridges during the ontogenesis of the basal telencephalic area. Some authors noted a third ridge during a certain period of development in, Homo: (His, 04; Hochstetter, '19; Kodama, '26; Källén, '51a), mouse: (Källén, 51a) and goat: (Ziehen, '06). In these reports, however, no unanimity exists about the position of such a transitory third

ridge, as can be seen in figure 4.

The preceding survey of the literature shows that many contradictory opinions exist about the development of the basal telencephalic region. In this chapter the morphogenesis of the ventricular ridges and their surrounding areas in the Chinese hamster will be analysed and an attempt will be made to find an answer to the following questions:

1. What is the morphological position of the ventricular ridges?
2. In which way do the two ventricular ridges originate?
3. Are there any indications for the existence of a third (transitory) ventricular ridge?
4. What is the final fate of the ventricular ridges?

4.3 *Observations*

In our analysis of the morphogenetic process we employed three-dimensional reconstructions of the rostral part of the CNS of a number of developmental stages. Drawings have been made after the models, showing (a) the outer surface and (b) the inner surface after the model was cut in the median plane. However, in older stages of development a sufficiently clear view on the inside of the lateral ventricle could not be obtained in this way. Therefore in the models of the brains of the older embryos a part of the diencephalon wall and nearly the whole medial hemisphere wall have been removed.

In developmental stages older than 16 days the lumen of the lateral ventricle is extremely narrow. In order to get a sufficiently clear view of the inside of the telencephalon wall of these stages, the sectioning of the models had to be performed in a complex way. However, the drawings of these sectioned models would be illegible. For this reason we employed the graphical reconstruction technique to visualize the ventricular relief of the lateral hemispheric wall, although being aware of the limitations of this technique.

In order to compare the results of both techniques, the inside view of the 16 days stage will be visualized by drawings of a sectioned model as well as a graphical reconstruction.

Finally it must be emphasized that the plexus choroideus, if present, has been deleted.

After having given a description of the morphology of the developmental stages E 12d+7h, E 12.5, E 13, E 13.5, E 14, E 16, E 18, PN 3 and the adult

stage, we shall try to give an overall picture of the morphogenesis and compare the observations with the data obtained from the literature.

The stage of 12 days and 7 hours

The first developmental stage to be described here shows slightly evaginated telencephalic hemispheres. The rostral part of the neural tube is shown in plate 1. The thin-walled neural tube has a simple form. Since the wall has nearly the same thickness at all places the surface relief of the outside of the wall shows an opposite aspect to that of the ventricular surface; that means that a groove on the outside corresponds to a ridge on the inside (cf. fig. 5). On top of the flexura cranialis the primordial mesencephalon is situated. Rostrally its boundary with the diencephalic area is indicated by a slight groove and ridge respectively. In the rostral part of the diencephalon three conspicuous structures can be observed. In the midline within the rostral limb of the flexura cranialis, the infundibulum can be recognized. In the lateral diencephalon wall there is an opening to the lumen of the optic stalk. The third structure which has to be mentioned here is a well formed groove or sulcus on the external surface of the prosencephalon partly in front of and dorsally to the optic stalk, the sulcus hemisphaericus or sulcus telodiencephalicus. On the inside, this sulcus corresponds to a curved ridge: the sulcus hemisphaericus ridge or torus hemisphaericus. Both sulci hemisphaerici of each side meet each other dorsally at a point where later on in development the velum transversum appears. More caudally they form the bottom of a wedge shaped space, which is situated between both hemisphere vesicles. This space is called "die Mantelspalte" (v. Mihalkovicz, 1877) or fossa interhemisphaerica (Ziehen, '06). Ventrally the sulci hemisphaerici fade away which corresponds on the inside with the course of the torus hemisphaericus towards the torus transversus. In the ventricular view the torus hemisphaericus and more rostrally the lamina terminalis border upon the presumptive foramen Monroi through which the ventricular space of the laterally evaginated hemisphere communicates with the medially situated part of the ventricle.

Finally it has to be emphasized, that at this stage of development the margin of the foramen Monroi, being the torus hemisphaericus, indicates a clear boundary between the telencephalon and the diencephalon. Ventrally, however, in front of the optic stalk this margin fades towards the torus transversus. As mentioned in chapter III, in our opinion the torus

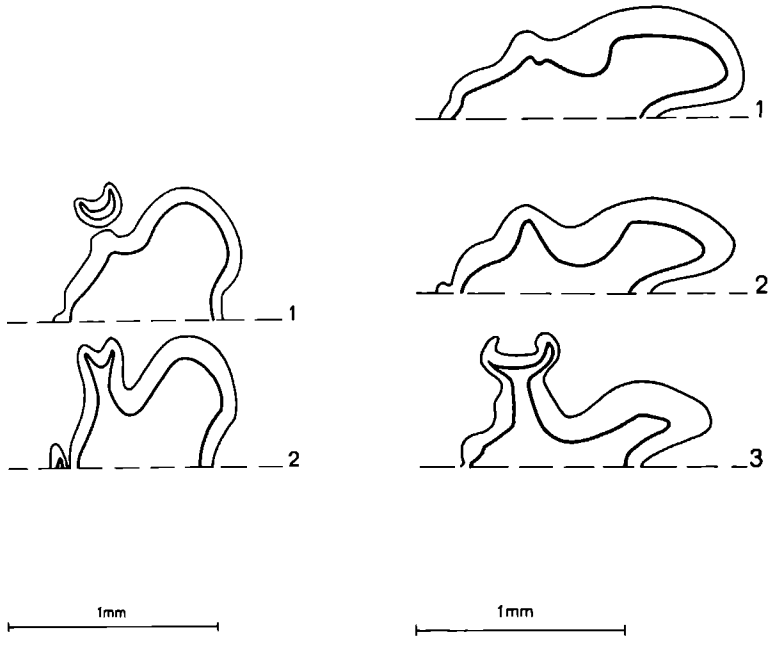

fig. 5 (Left.) Horizontal sections showing the relations between the inner and outer surfaces of the prosencephalon at the stage E12+7h. The levels of the sections are indicated in plate 1.

fig. 6 (Right.) Horizontal sections showing the relations between the inner and outer surfaces of the prosencephalon at the stage E12.5. The levels of the sections are indicated in plate 2.

transversus, recognizable as a small thickening in the midline must mainly be considered as a telencephalic structure, forming the basal telodiencephalic boundary. In this respect we agree with v. Mihalkovicz (1877), Tandler et al. ('07, '15), Hines ('22), Kuhlenbeck ('54) and Keyser ('72).

The stage of 12.5 days

The next developmental stage which we will describe is represented by an embryo of 12.5 days post conception. Comparing plates 1 and 2, one of the main differences with regard to the morphological pattern of the neural tube is the expansive outgrowth of its rostral parts. Besides the ventricular relief is much more accentuated than in the former stage. Especially in the diencephalic area it shows a number of bulge-like

structures corresponding with the neuromeres. The significance of these structures in respect to the development of the diencephalon has been investigated by Keyser ('72). In the lateral diencephalic wall the optic stalk is still open; the optic cone is very obvious in this period of development. However, the most important change is the appearance of a slight hill-like structure situated in front of the optic stalk opening. It is a typical ventricular structure, the external surface shows nothing of it. Therefore in our terminology it is called the ventricular elevation (plate 2). As can be seen in figure 6 this elevation is a single structure which cannot be subdivided into two or more parts and is characterized by a local thickening of the ventricular wall. It is situated in the floor of the foramen Monroi, mainly extending forward and protruding into the lateral ventricle, whereas caudally the elevation reaches as far as the opening of the optic stalk. In other words the ventricular elevation must be considered as a structure derived mainly from the lateral hemisphere wall and partly from the wall of the diencephalon. From the morphological point of view the consequence of the formation of the ventricular elevation is bipartite:

1. a considerable change of the form of the foramen Monroi;
2. the "disappearance" of the basal part of the torus hemisphaericus.

The ventricular elevation rides on this torus with a short limb extending in the wall of the third ventricle and a longer massive one extending in the wall of the lateral ventricle. Due to this last change the more or less sharp margin of the torus hemisphaericus has disappeared in the basal part of the telodiencephalic transitional area. The problem of the telodiencephalic boundary will be discussed later in more detail.

Finally, the advance of the development of this developmental stage as compared with the preceding one can be summarized as follows:

- a remarkable increase in size of the rostral parts of the neural tube has taken place,
- at the basal telodiencephalic area a ventricular elevation has developed.

The stage of 13 days

The next stage is represented by an embryo of 13 days post conception. Plate 3 shows the ventricular surface from medial view, whereas plate 4 presents a view from above. In order to give an overall picture of the ventricular relief of the lateral hemisphere wall, the medial hemisphere wall has been depicted as translucent.

A very important change in the neighbourhood of the ventricular elevation within the prosencephalon can be observed. We will now focus our attention on this area.

Reference to plates 3 and 4 shows that a relatively wide, slightly curved plane borders the rostral part of the ventricular elevation. The significance of this plane is, that it indicates the appearance of a second elevated part of the wall. It is important to note that in this stage the plane is entirely separated from the ventricular elevation as shown in figure 7. The delimiting groove between these two parts is called the sulcus subpallii intermedius. It is a deep groove passing from the bottom of the hemisphere vesicle up to a dorsal and a somewhat caudal position and ending on a level at which the hemisphere is thin-walled and the ventricular relief is curved in a concave direction as the remaining part of the hemisphere vesicle. In other words: caudal to the hemisphere part of the ventricular elevation and to the plane no ridge-like structure exists. This is important with regard to later development.

It must be emphasized here that on account of the slight morphological difference between this plane and the surrounding concave hemispheric area, it is only possible to recognize and to bound it by making use of material which is sectioned in a carefully selected direction. Already Keyser ('72) has called attention to the perceptibility of relief structures with regard to the direction of sectioning: ".....only those prominences and excavations are in an optimal position for observation, which are oriented at right angles to the plane of sectioning" (p. 43). In our opinion this fundamental statement should always be remembered when studying relief structures.

Let us now go back to the ventricular relief of the 13 days stage. It is clearly to be observed that the plane structure must be considered as a derivative of the hemisphere wall. Rostrally it is bordered by a shallow groove called the sulcus subpallii dorsalis. The basal boundary, which in later development will be marked by a groove, is also very vague. The ventricular elevation forms the bottom of the foramen Monroi (plates 3, 4). Its caudal boundary is formed by a groove called the sulcus intraencephalicus anterior (v. Kupffer, '06). This sulcus is running from the still very wide foramen Monroi to the opening of the optic stalk. The area situated directly rostrally to this sulcus is the primordium of the preoptic region. It is clearly visible that this region must be considered as belonging to the ventricular elevation.

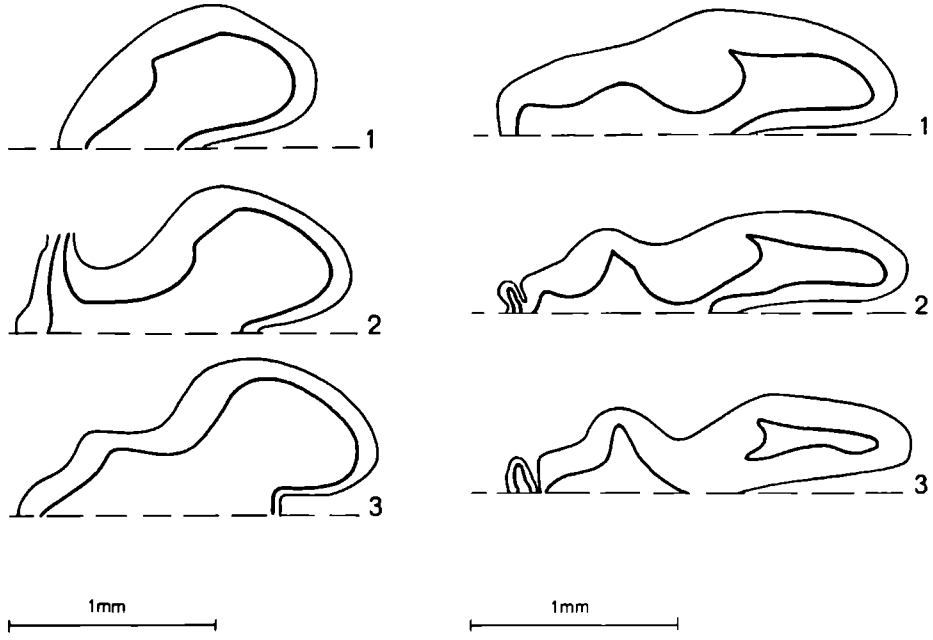

fig. 7 (Left.) Fronto-horizontal sections showing the relations between the inner and outer surfaces of the prosencephalon at the stage E13. The levels of the sections are indicated in plate 3.

fig. 8 (Right.) Horizontal sections showing the relations between the inner and outer surfaces of the prosencephalon at the stage E13.5. The levels of the sections are indicated in plate 6.

Summarizing it can be noted that shortly after the ventricular elevation has appeared, lateral to it a plane structure develops which is entirely separated from the elevation. The significance of this plane will be explained in the next stage of development.

The stage of 13.5 days

This developmental stage will be described with the aid of plates 5-8. In plate 7 parts of the diencephalon- and telencephalon wall are removed in order to get a better view on the ventricular relief of the lateral wall of the hemisphere. In plate 8 only the main part of the basal area of the forebrain is shown.

The expansive growth of the rostral parts of the brain tube has proceeded.

Thereby the depth of the flexura cranialis is increased. Comparing plates 2 and 5 it is obvious that the diencephalon wall is covered more and more by the laterally situated hemispheres. Besides the outside shape of the hemispheres has changed from a sphere-like structure into a more oval one. The enormous outgrowth of the hemispheres leads to the deepening of the fossa interhemisphaerica.

The ventricular surface of the diencephalon is still characterized by neuromere structures. More rostrally the optic stalk is closed and the remaining deep pit on the inside is called the recessus opticus. Plate 6 shows that the spatial relationships in the neighbourhood of the foramen Monroi have changed considerably. Due to the outgrowth of the ventricular elevation, which from now on will be called medial ventricular ridge (see later), the foramen Monroi has narrowed. On the bottom of the opening, the margin is formed by a small threshold-like structure which connects the medial ventricular ridge with the torus transversus (plate 7). This connection is also shown in figure 8 section 3. Besides this figure shows the relation between the ventricular ridge and the external sulcus hemisphaericus. Especially in the sections 2 and 3 of this figure it is clear that the ridge has an extension in the wall of the third ventricle, whereas a close relation exists with the optic recess. The sulcus intraencephalicus anterior forms the caudal border of the ridge. This sulcus runs dorsally to the foramen of Monro and from here it runs caudally and somewhat laterally within the lateral ventricle as sulcus terminalis (cf. plates 7 and 8).

Observing the ventricular relief of the lateral hemisphere wall it is obvious that a second ventricular ridge has developed. This ridge is situated mainly lateral and rostral to the former ridge and corresponds with the "slightly curved plane" described in the preceding stage. The ridges are for the greater part separated from each other by the sulcus subpallii intermedius. This sulcus corresponds with the fissura intercruralis of His ('04), the fissura neopalaeostriatica of Ariëns Kappers ('23), the "strio-caudate" sulcus of Johnston ('23) and the interstriatal sulcus of Brown ('67) and Humphrey ('68). As can be seen in plate 7 this sulcus runs from the bottom of the hemisphere up to a dorsal and somewhat caudal level. However, the caudal parts of both ridges are not separated by this sulcus (plate 8). In this region we observe an undivided part of the elevation. The question now is in which way this part originates: must it be considered as a "new" structure developing more or less independent caudal to the two ridges or does it come about by

fusion of the caudal parts of both ridges? Although the latter possibility seems to be the most likely one, morphologically the first one cannot be excluded. Only an investigation of the histogenesis in this region may provide an answer (see chapter V). Nevertheless, the caudal pole of the ventricular elevation seems to be a "transition area" between the two ridges. This is shown very clearly in plate 8.

The lateral ventricular ridge is dorsally bounded by a groove which can be identified as the sulcus subpallii dorsalis described in the former stage. Dorsally to this boundary the still thin-walled concave part of the hemisphere is situated. The sulcus itself is a shallow groove, reaching caudally up to a small pit-like extension of the lateral ventricle, which can be recognized as the first appearance of the cornu inferius.

In comparison with the former stages, the most important morphological differentiations can be summarized as follows:

- the closure of the optic stalk and thereby the origin of the optic recess;
- the appearance of a second (lateral) ventricular ridge within the lateral hemisphere wall, corresponding with the "plane structure" described in the preceding stage;
- the appearance of a caudal, undivided part of the ventricular elevation, which connects the two ventricular ridges.

The stage of 14 days

The expansive outgrowth of the rostral parts of the brain in this stage is shown in plates 9, 10, and 11. This can be observed very well on the telencephalic hemisphere, which covers nearly half the diencephalon wall. The flexura cranialis is changed into a narrow cleft. The landmarks, as described in chapter III, are well differentiated now and can be clearly observed. The diencephalic wall still shows a neuromeric pattern as in previous stages. However, in some areas a local thickening of its wall appears. In plate 10 such an area can be seen dorsal to the boundary of the foramen Monroi. This thickening is called the eminentia thalami; it corresponds with a part of the torus hemisphaericus of earlier stages. Although a clearly visible optic recess exists (plate 10), the optic stalk is transformed into a solid strand of nervous tissue: the anlage of the optic nerve (plate 9). The optic cone which was sharply bordered from its surrounding areas in former developmental stages, is now entirely "incorporated" within the diencephalic wall.

Plate 10 shows that the foramen Monroi seems to be closed. Only a narrow elongated space persists at the dorsal and medial side of the medial ventricular ridge. As in earlier stages the medial part of this ridge is continuous with the wall of the third ventricle. In plate 11 this relation is clearly shown. Due to an expansive outgrowth the ventricular ridges now protrude far into the lumen of the lateral ventricle. A part of the medial ventricular ridge is situated rostral to the level of the lamina terminalis. It seems as if during development this ridge "runs more and more rostrally" (Källén '51a). However, it must be kept in mind that the position of the lamina terminalis is not a static one, since the relations between the various parts of the brain wall are continuously changing. Rostrally, the two ventricular ridges are still separated from each other by the sulcus subpallii intermedius. Caudally however, a large undivided protruding part can be observed. In comparison with the former stage, this part has increased much in size. Probably, this increase is due to a partial disappearance of the sulcus subpallii intermedius.

Summarizing the main progressions in development it can be noted that:

- the diencephalon is covered more and more by the expanding cerebral hemispheres;
- the outgrowth of the medial ventricular ridge has transformed the foramen Monroi into a narrow, elongated cleft;
- the ventricular ridges seem to have shifted into the telencephalic region, although at this developmental stage a wide continuity persists with the wall of the third ventricle.

The stage of 16 days

In this developmental stage the external diencephalic wall is almost entirely covered by the hemispheres, as shown in plates 12 and 13. The anlage of the epiphysis is situated between the two hemispheres. The anlage of the bulbus olfactorius can be observed at the rostral end of the hemisphere. The increase in volume of the areas surrounding the foramen of Monro causes a further narrowing of this opening.

Comparing the midline structures with those of the preceding stage, it can be noted that the shape of the preoptic recess is changed, because the angle between the torus transversus and the chiasma opticum has sharpened. Both structures last mentioned have increased in thickness, whereas in the torus

transversus the first anlage of the commissura anterior can be recognized.

The extension of the ventricular ridges in the lateral ventricle is shown in plates 14 and 15. Rostrally both ridges are still separated by the sulcus subpallii intermedius. The caudally situated, undivided part of the eminence has much increased in size as can be observed in plates 15 and 16. Medially this part is bounded by the curved sulcus terminalis. This sulcus is running from the foramen Monroi to the caudally situated cornu inferius of the lateral ventricle. The sulcus subpallii dorsalis, which appears for the first time in development as a sharp groove, also ends in the cornu inferius.

As mentioned before, the opening of the foramen of Monro is much reduced in comparison with earlier stages. The consequence is that the medial part of the ventricular ridge which extends in the wall of the third ventricle, seems to get isolated from the rest of it. However, it must be kept in mind that this area, being the preoptic region, which is situated in front of the sulcus intraencephalicus anterior has to be considered as belonging to the original ventricular elevation.

In comparison with the former stage the following transformations can be noted:

- the gradual closing up of the foramen Monroi;
- the gradual "isolation" of the medial part of the medial ventricular ridge, being the preoptic region.

As mentioned before, the lateral ventricle becomes narrower in later stages of development. For this reason it is impossible to remove parts of the wall in our models in a simple way in order to get an inside view of the lateral ventricle. The alternative way to study this area is to employ graphical reconstructions (cf. plates 14 and 16). The later phase of development has been studied with the aid of such reconstructions (plates 17, 18, 19).

The stage of 18 days

Plate 17 shows the ventricular relief of the lateral hemisphere wall plus the preoptic region of the developmental stage of 18 days post conception. In this medial view the midline structures are also indicated.

The small foramen of Monro is rostrally bound by the extensively outgrowing torus transversus. Within this last formation two important commissural fibre systems can be clearly recognized. These are the anlagen of the corpus callosum (including the commissura hippocampi) and the

commissura anterior, which was already present at the 16 days stage. Dorsally the roof of the diencephalon is formed by the irregularly folded plexus choroideus, connecting the epiphysis with the torus transversus region. Rostrally, the lumen of the lateral ventricle extends into the olfactory bulb. Caudally, the cornu inferius of the lateral ventricle has increased in size. Concomitantly, the area ventral to the sulcus terminalis ("Hemisphaerenstiel" of His, '04, cerebral or hemisphere stem of J.E. Rose, '42) shows an enormous outgrowth by which the curvature of the sulcus itself increases considerably. At the foramen Monroi the sulcus terminalis is continuous with the sulcus intraencephalicus anterior. The latter passes over the wall of the third ventricle toward the region of the preoptic recess. The optic recess has almost disappeared, whereas the sulcus intraencephalicus anterior is transformed into a shallow groove. In comparison with previous stages the ventricular relief of the lateral hemisphere wall shows a conspicuous change in the proportions between the elevated area and the dorsally situated pallial region. The explosive outgrowth of the pallial area causes a fading of the sulcus subpallii dorsalis; thus the boundary between the pallium and the ventricular ridge area becomes less distinct.

In the ventricular ridge area itself the sulcus subpallii intermedius has entirely disappeared. This means that the ventricular ridges, which originally develop as two separated morphological entities, are completely transformed into a single ventricular eminence. By way of the bottom of the foramen Monroi, the ventricular eminence still holds a direct relation with the preoptic region.

The developmental progress as observed in the 18 days stage can be summarized as follows:

- the anlage of the corpus callosum appears;
- the pallial region has expanded considerably;
- due to the disappearance of the sulcus subpallii intermedius the two original ventricular ridges have completely united into a single ventricular eminence;
- the cerebral stem area expands, which leads to the formation of the cornu inferius of the lateral ventricle,

The stage of 3 days postnatal

The ventricular relief at the stage of 3 days postnatal is shown in plate 18.

Generally it can be said that most midline structures display an extensive outgrowth. This concerns both the bottom and the roof area of the mesencephalon (the tegmentum and tectum mesencephali respectively) and also the optic chiasm plus the torus transversus region of early stages. Within this last structure the increase is related to the development of the corpus callosum and the commissura hippocampi and also to the outgrowth of an area connecting both hemispheres: the septal area. In the middle of the third ventricle parts of the thalamic region of both sides meet each other in the midline and fuse, forming the massa intermedia.

Looking at the ventricular surface of the lateral hemisphere wall rostrally a narrow extension of the ventricular lumen can be observed, reaching the olfactory bulb region. Caudally the further development of the cornu inferius of the ventricle coincides with the increase in mass of the cerebral stem area. Besides the thickness of the wall of the pallial region is increased. The expansion of both areas results in a reduction of the ventricular space in a dorso-ventral direction.

The shape of the ventricular eminence has changed considerably. It now consists of a main dorsal convex part and a smaller flat ventral part. The dorsal part looks like a curved elongated ridge, which is wide rostrally and gradually tapers toward the cornu inferius. It is dorsally bordered by the sulcus subpallii dorsalis. The ventral part is medially bordered by the angulus ventralis in front of the foramen of Monro and behind it by the sulcus terminalis. As compared with the former stage the proportion between the ventricular eminence and the ventrally situated cerebral stem region is obviously changed in favour of the latter. This suggests that the ventricular eminence partly contributes to the expansion of the cerebral stem region.

In the rostral and basal wall of the third ventricle, the sulcus intra-encephalicus anterior remains as a vague structure. The preoptic region in front of it retains also in late development a relation to the elevated area of the lateral hemisphere wall by way of the foramen Monroi. As mentioned before, it must be considered as a derivative of the medial ventricular ridge.

The morphological changes observed in this stage as compared with the preceding one are:

- the progressive development of a number of midline structures has caused an increasing thickness of the wall; the expansion of the previous torus transversus area is notably due to the development of the corpus callosum (and commissura hippocampi) and the septal area situated between both

hemispheres;
- the changes in shape of the remaining part of the ventricular eminence suggest a participation of the eminence in the outgrowth of the cerebral stem area.

The adult stage

It has already been known for a long time that after birth the development still goes on. This also holds for the brain region. In order to obtain a complete view of the morphogenesis of the lateral hemisphere wall, we will finally describe the adult stage, i.e. at the age of 100 days postnatally.

Generally it can be said that after birth the midline structures increase in volume. This holds true in particular for the mesencephalic wall, the optic chiasm and the septal area (see plate 19). In comparison with the 3 days postnatal stage the optic chiasm reaches more rostrally in respect to the septal area, whereas the corpus callosum shows a caudal expansion. In the adult stage the last structure covers the main part of the plexus chorioideus of the third ventricle. The space between both structures belongs to the extracerebral space as indicated in plate 19. Caudal to this space the epiphysis is situated, connected with the roof of the diencephalon by an elongated stalk.

The ventricular relief of the lateral hemisphere wall in the adult, shows some notable changes. First of all the rostral extension of the ventricular space within the olfactory bulb appears to have disappeared. The lateral ventricle can be subdivided into three parts: the anterior horn, situated in front of the foramen of Monro, the central part, situated behind it, and the inferior horn (cf. Westergaard, '70).

The lumen of the lateral ventricle is very narrow and in some areas even the medial hemisphere wall is fused with the lateral one. These areas of fusion have been called coarctationes ventriculi by Westergaard ('68, '70), who demonstrated that in several adult mammalian species (mice, rats, hamsters, guinea pigs, rabbits) such coarctations exist. This means that their presence must be considered as a normal phenomenon in the adult mammalian brain.

In the adult stage the ventricular eminence can be subdivided into two parts, which are comparable to those observed in the 3 days postnatal stage. The ventral flat part, however, is wider, especially in front of the foramen of Monro. Besides it is visible that the cerebral stem area has further expanded, causing an increased curvature of the ventricular eminence.

The sulcus intraencephalicus anterior persists in the adult stage as a shallow groove. The preoptic region is almost isolated from the ventricular eminence. This is also due to the extreme narrowing of the foramen of Monro.

As compared with the 3 days postnatal stage, the final morphological differentiations (taking place after birth) are:

- a continued increase in volume of a number of midline structures;
- the disappearance of the ventricle of the olfactory bulb;
- the development of the so-called coarctation areas;
- the continued increase of the cerebral stem area, which puts its stamp on the definitive shape of the lateral ventricle.

4.4 *Discussion*

With the aid of the observations on the morphology of a number of closely graded developmental stages, we are able to discuss the morphogenetical process of the prosencephalon. Besides an answer can be given on a number of problems existing in literature, as mentioned at the beginning of this chapter.

The discussion will be subdivided into two parts: first we will focus our attention on the morphogenesis of the ventricular ridges themselves and, secondly, the transformations of the areas surrounding the ridges will be regarded.

4.4.1 *The development of the ventricular ridges*

The first developmental stage which has been described, has a very simply formed neural tube in which the thin wall shows a ventricular relief that in an opposite way corresponds to that of the external surface: "... stellt der innere Hohlraum ein genaues Negativ der äusseren Hirnform dar" (Kahle, '69, p. 6). For this reason the external sulcus hemisphaericus situated in the rostral part of the prosencephalon evokes the torus hemisphaericus on the inside. According to the opinions of v. Mihalkovicz (1877), Tandler et al. ('07, '15), Hochstetter ('19), Kuhlenbeck ('54, '56) and Keyser ('72), this torus indicates the telodiencephalic boundary in early development.

In the first phase of development of the neural tube, the extension of the prosencephalon occurs with maintenance of more or less the same form; this means that the outgrowth mainly takes place in a tangential direction. This mode of extension is changed when within the prosencephalic wall the an-

lage of a ventricular elevation appears. Situated in front of the optic stalk and basal to the primordial foramen of Monro this elevation extends in the lateral hemisphere wall as well as in the third ventricular wall. As a result the basal part of the torus hemisphaericus, and thus the morphological indication of the telodiencephalic boundary, fades away. This means that the boundary between the primordial telencephalon and diencephalon only exists for a short time and gradually disappears by the development of the ventricular elevation. Concerning the localization and the extension of the ventricular elevation, it can therefore be noted that the first sign of the elevation is situated in the region of the hemispheric wall as well as in that of the diencephalon. It must be stressed here that this opinion is in harmony with the definition of the telodiencephalic boundary - being the torus hemisphaericus - (cf. Vaage, '69). In other words, if this boundary should be situated more caudally (His, '04; v. Kupffer, '06; Ariëns Kappers, '23, among others) the whole elevation could be considered as a telencephalic structure.

Quite another opinion about the origin of the ventricular ridges is given by Grünthal ('52). He considered the ventral part of the hemisphere as an outgrowing part of the diencephalic hypothalamic region, whereas the dorsal hemispheric part would be constituted by a thalamic outgrowth. Accordingly, he concluded that the elevation is a derivative of the diencephalon, which is subsequently shifting forward to get a telencephalic position. This is in contradiction to our view as mentioned above. It must be stated here that in our opinion it is not possible to distinguish a primordial thalamic and hypothalamic area before the subdivision of the prosencephalon into the telencephalon and the diencephalon has taken place. Besides Grünthal was not able to provide evidence for the displacement of the elevation during development which he suggested. It is evident from plate 2 and figure 6 that the main part of the anlage of the elevation must be considered as a derivative of the basal telencephalic wall (regarding the position of the ridge to the external sulcus hemisphaericus) since it originates after the endbrain vesicle has been formed (cf. plate 1 and fig. 5).

At the beginning of this chapter we mentioned still another opinion about the origin of the elevation. Tandler and co-workers ('07 and '15) considered the developing ridge as a lateral extension of the torus transversus. However, the ridge and the torus region appear to be separated by a small depressed area (cf. plate 2); thus there is no direct relation between the ridge and

the torus transversus. On that account it is hardly possible to interpret the elevation as a laterally situated derivative of the torus transversus, as was also noted by Hochstetter ('19).

Let us turn now to the further development of the ventricular ridge area. Soon after the appearance of the ventricular elevation, lateral to it a part of the hemisphere wall changes into a slightly curved plane which is separated from the elevation by a shallow groove. Since this plane area must be considered as the first appearance of a second elevation, it can be noted that originally both structures morphologically form two entirely separated entities. This view is in contradiction to the opinion of some other authors, who suggested that the two ridges originate by a subdivision of one single ridge (Tiedeman, 1816; v. Mihalkovicz, 1877; Hochstetter, '19; Källén, '51a and Hamilton et al., '72). On the other hand our observations are in agreement with those of other investigators, who emphasized that both ridges do not originate at the same time (Kodama, '26; Grünthal, '52; Hewitt, '58, '61; Brown, '67; Kahle, '69). These authors stated that the first appearing slight elevation at the level of the foramen of Monro constitutes the primordial medial elevation. We affirmed this statement (cf. plates 4 and 8) as opposed to Humphrey ('68), who considered that elevation as the primordial lateral ridge.

Both ridges expand during further development, protruding more and more into the lumen of the lateral ventricle. The expansion of the medial ridge contributes to an increased narrowing of the foramen Monroi, which at the end of the development is reduced to a small, cleft-like opening. Although both ridges originally appear as two completely separated entities, it can be observed that they unite during development, starting at the caudal pole of the hemisphere. This unification proceeds anteriorly and finally no subdivision can be made any more within the ventricular eminence (cf. plates 11, 14, 16 and 17). Ariëns Kappers ('23) and Johnston ('23), however, described that in the adult human brain a subdivision within the eminence area remains, but most authors have rejected this opinion.

As mentioned before some authors observed within the total elevated area, during a certain period of development, a subdivision into three parts. His ('04) and Ziehen ('06) suggested this in early developmental stages. Hochstetter ('19), Kodama ('26) and Källén ('51a) observed the existence of a third ridge in later developmental stages although it must be taken into account, that its position varies in all three reports (cf. fig. 4). Our observations on Chinese

hamster material provide no evidence for the existence of a third elevation, neither in early nor in later stages of development.

4.4.2 *The morphogenesis of the ridges in relation to the surrounding areas*

The progressive extension of the structures bordering the primitive foramen of Monro being the eminentia thalami, the torus transversus and the medial ventricular ridge, causes an increased narrowing of that opening. This implies that the diencephalic ventricular surface area of the medial ventricular ridge becomes progressively isolated from its, more extensive, telencephalic surface area. However, it must be emphasized that it is not possible to define a plane indicating the telo-diencephalic border. Although in early developmental stages many authors reported an extension of the ridge within the third ventricular wall (v. Mihalkovicz, 1877; His, '04; Hochstetter, '19; Hines, '22; Källén, '51a; Bartelmez and Dekaban, '62; Humphrey, '68) they did not clearly describe the final fate of this extension. Therefore it is stressed that in our opinion the ventricular surface of the preoptic region and its derivatives must be considered as originally derived from the medial, ontogenetically oldest, ventricular ridge. Caudally this region is bordered by the sulcus intraencephalicus anterior. The persistance of this sulcus up to adulthood agrees with the observation of Vaage in the chick ('69).

In the description of the morphology of the various developmental stages we have already indicated the close relationship between the ventricular eminence and the cerebral stem region. The ventricular eminence seems to be implicated in the extension of the cerebral stem region, as was suggested earlier by v. Mihalkovicz (1877) and Grönberg ('01). During development the expansion of the medial part of the ventricular eminence, together with the extension of the cerebral stem itself, effectuate a broader communication with the diencephalon. This evokes an elevation of the sulcus terminalis and results in an increase of the curvature of that sulcus as well as of the dorsal part of the eminence (cf. plates 16-19).

Finally, we will call attention to the changing proportions between the elevated area and the dorsally situated pallial region. It can be noted, that up to the 16 days stage of development inclusive, the latter region has a more or less thin-walled character. Up to that time its outgrowth mainly occurs in a tangential direction, i.e. parallel to the ventricular surface. The extension of the elevated area principally shows a radial outgrowth tendency from the

beginning, i.e. perpendicular to the ventricular surface. In later developmental stages a strong radially directed expansion of the pallial area can be observed. Thereby the proportions between the structures constituting the basal and dorsal part of the lateral hemisphere wall are transformed considerably (cf. plates 17, 18 and 19).

4.4.3 *Conclusions*

A number of figures made after both spatial and graphical reconstructions facilitated the study of the morphogenesis of various developmental stages. From the observations made in this study the main course of the morphogenesis can be deduced.

Our attention was focussed on the morphogenesis of the ventricular ridges, which originate in the prosencephalic region, in relation to the surrounding areas. Summarizing the following conclusions can be drawn:

1. The first appearing ventricular ridge which develops in the floor of the foramen of Monro and thereby obscures the telodiencephalic boundary is the medial ventricular ridge.
2. The lateral ventricular ridge originating at a later period of development must be considered as a derivative of the at that time still thin-walled telencephalic hemisphere.
3. Originally the two ventricular ridges are entirely separated; soon after the appearance of the lateral ridge, they gradually merge into one ventricular eminence in which no subdivision can be observed any longer.
4. The preoptic region is a derivative of the medial ventricular ridge.
5. The ventricular eminence is closely implicated in the outgrowth of the cerebral stem region.

5 THE MATRIX LAYER

5.1 *Introduction*

In the previous chapter a description is given of the morphogenesis of the ventricular surface of the lateral telencephalic wall and the rostral part of the diencephalic wall. Along the ventricular surface the matrix layer is situated, which must be considered as the source of the cells which gradually constitute the mantle layer, situated peripheral to the matrix layer.

This developmental feature emphasizes the important intermediate position of the matrix layer. On the inside the matrix borders the ventricular lumen and therefore is directly implicated in the morphogenesis of the ventricular surface. On the outside the matrix is involved in the development of the mantle layer, which means that the matrix also has a direct relation with the histogenesis.

After an explication of the terminology to be used in this chapter, a brief review will be given of the literature concerning the main aspects of the development of the matrix. Subsequently we will describe the method and the techniques which we have used to study and to visualize the condition of the matrix in the various developmental stages. These introductory notes are followed by a description of our observations. Finally, our findings will be discussed in view of the data obtained from the literature.

5.2 *Terminology*

As early as 1904 His discerned three layers within the embryonic human neural tube, which he termed from the inside outwards: "Innenplatte" or "Matrix", "Mantelschicht" or mantle layer and "Randschleier" or marginal layer. According to the present day knowledge of the developing vertebrate central nervous system, this classic concept has to be modified, at least when the telencephalon is concerned, because of the existence of a fourth layer between the matrix and the mantle layer. This fourth zone was first demonstrated in adult and late developmental stages and was called subependymal layer (Allen, '12; Globus and Kuhlenbeck, '44; Smart, '61; Noetzel and Roux, '64). It corresponds with the "Keimlager" of Kahle ('51, '69). However, since its equivalent is also present in younger developmental stages, in which no ependyma exists as yet, we introduce the name "submaternal layer". This term

is applied in order to stress the functional aspect of this layer, which forms a secondary proliferative compartment. In 1970 the Boulder Committee suggested a revised terminology with regard to the four fundamental zones of the developing CNS. They proposed the names: "ventricular zone" (instead of matrix), "subventricular zone" (subependymal or submaternal layer), "intermediate zone" (mantle layer) and "marginal zone". However, some objections can be made against this purely topographical terminology. First, the dynamic generative aspect of both the matrix and the submaternal layer is entirely lost. Secondly, the mantle layer indeed holds an intermediate position, but at first between the matrix and the marginal layer and subsequently between the submaternal layer and the marginal layer. Thirdly, the term "intermediate zone", whenever used, both topographically and histogenetically would be more applicable to the submaternal layer. For these reasons the classical terms: matrix, mantle layer and marginal layer completed with "submaternal" layer will be employed in the present study.

In the sequence of their appearance these four zones can be briefly characterized in the following way:

1. The matrix layer is a periventricularly situated structure, consisting of mitotically active elements, which during their generation cycle show an elevator movement of their nuclei to and from the ventricular surface. The constituent matrix cells are the precursors of neurons and macroglial cells of the central nervous system. The matrix layer is a transitory structure which gradually looses its proliferative activity. Eventually the matrix disappears, possibly by being transformed into the so-called ependymal layer. The ependyma, being a monocell-layered epithelium without any mitotic activity, ultimately lines the ventricle.
2. The marginal layer is a cell-free zone which develops on the outside of the matrix layer. The marginal layer initially consists of the outer cytoplasmic processes of the matrix cells. In later development these processes are gradually substituted by ingrowing axons and dendrites. This composition of the marginal layer is retained up to the adult stage, in which the marginal layer is still recognizable as the outermost zone of the central nervous system.
3. The mantle layer is the third zone to develop in a position intermediate between the matrix and the marginal layer. Initially the mantle layer is constituted by neuroblasts and glioblasts which after their final mitotic

division leave the matrix layer. Subsequently, also neuronal, macroglial and possibly microglial elements generated by the submaternal layer are added to the mantle layer. After their entrance into the mantle layer the neuroblasts and glioblasts eventually migrate to their ultimate position and differentiate into mature neurons and macroglial cells, respectively. During further development the mantle layer is also supplied with mesodermal derivatives such as blood vessels and probably microglia. The mantle layer gradually increases in thickness pari passu with the decrease of the matrix layer. Finally, at the end of development, the mantle layer has acquired a predominant position within the central nervous system.

4. The submaternal layer is a very conspicuous structure in the telencephalon, although it might also be present in other regions of the central nervous system. The submaternal layer arises late in development, between the matrix and the mantle layer. Its constituent cells differ from mantle layer cells by being mitotically active like the matrix cells, but contrary to the latter their nuclei do not exhibit an elevator movement to and from the ventricular surface during their mitotic cycle. Although much less is known about the submaternal layer as compared with the other three fundamental layers, evidence has been provided that submaternal cells probably give rise to both neurons and macroglia cells. Possibly microglia cells also originate in this layer. The submaternal derivatives just mentioned enter the mantle layer to differentiate, whereas the remaining part of the submaternal cell population retains its mitotic activity. The submaternal layer persists up to the adult stage in which it is present just peripheral to the ependymal layer.

It should, however, be emphasized that in this chapter, in which the development of the matrix layer is analysed, for practical reasons the presence of a submaternal layer is left out of consideration. After this terminological introduction we will now give a brief survey of the data obtained from the literature concerning the matrix layer.

5.3 *Literature*

5.3.1 *The constituent cells of the matrix layer*

The first concept concerning the cellular constituents of the matrix was published by His (1889, 04). In his opinion the cell elements had to be subdivided into a group of germinal cells ("Keimzellen") and a group of spongioblasts, which would give origin to neuroblasts and glioblasts, respectively.

Within the matrix layer the spongioblasts would form a framework ("Markgerüst") in which the "Keimzellen" were situated. Some authors however, among them Kölliker (1896) and Schaper (1897), believed that the matrix layer consisted of cell elements belonging to one single cell type varying in shape because they were in different phases of the mitotic cycle. The great authority of His in neuroembryology, has led to the general acceptance of his concept of two different cell populations, for a long time.

In 1935 the studies of F.C. Sauer caused a revolution concerning the matrix concept although most of his ideas were in harmony with those of Schaper (1897). Sauer conclusively showed that His's interpretation of the cell elements of the matrix was not correct. Besides he presented a clear description of the kinetics of the matrix cells, which will be elaborated in the following paragraph.

The rediscovery of the matrix concept of Schaper (1897) seems to put an end to the contradictions about the matrix construction, since a great number of publications confirmed Sauer's view (Watterson e.a., '56; M.E. Sauer and Chittenden, '59; M.E. Sauer and Walker, '59; Sidman e.a., '59; Fujita, '62, '63, '64, '66; Watterson, '65; Langman, '65; Atlas and Bond, '65, among others). However, the homogeneous cell population of the matrix layer was questioned by Stensaas and Stensaas ('68). Referring to the autoradiographical study of Fujita ('63) they stated: "By itself the observation that all cells are eventually labeled does not rule out the presence of more than one cell type" (p. 360). On the basis of Golgi impregnation and electronmicroscopic investigations they noted that within the matrix layer three types of cell elements could be discerned: columnar epithelial cells, neuroblasts and spongioblasts. More recently Rakic ('72) described in older monkey embryos two types of cell somas within the matrix layer of the primordial pallial region. Studying the migration mechanism of the neuroblasts in the mantle layer, he provided evidence that these cells make use of a radially oriented fibre system. These fibres would belong to a distinct transient class of glial cells, which in young specimens would be situated predominantly in the matrix and submaternal layers, whereas lateron in development they would shift outwards.

From this brief review of the literature concerning the cell elements of the matrix it may be concluded that the question whether or not these cells belong to a single type still remains open.

However, it should be pointed out that both opinions are less incompatible than they seem to be. It might well be that, originally, all matrix cells be-

long to a single homogeneous cell population, whereas at a later time of development two or even more different cell types are present.

5.3.2 *Kinetics of the matrix cells*

Although the matrix cells are randomly distributed along the mitotic cycle, Sauer ('35) demonstrated that the various mitotic phases are bound to a particular topographical position within the matrix layer. Since metaphases always occurred at the ventricular surface and interphases in the outer zone of the matrix, he concluded to a movement of the nuclei of the matrix cells to and from the ventricular surface within this layer. This cyclic movement of the cell nuclei is called the elevator movement and evidence in favour of this movement has been adduced by a number of investigations (autoradiographic technic: Fujita, '62; Sidman et al., '59; quantitative determination of DNA contents of the cells: M.E. Sauer and Chittenden, '59). In the outer zone of the matrix the DNA-content of the cells is duplicated. Then the cells move to the ventricular surface where they divide into two daughter cells. These daughter cells migrate back to the outer zone of the matrix, preparing the next division. The time between two successive divisions of the cell is called the generation time. Several authors among them Fujita ('62) and Langman e.a. ('66) demonstrated that with the progress of development a prolongation of the generation time occurs. This means that the proliferation activity of the matrix, which we will call the intrinsic matrix activity, decreases with increasing development. However, some indications exist, suggesting that the kinetics of the matrix cells is not that simple. Bergquist and Källén ('54) believed that in some telencephalic and caudal thalamic areas the proliferation activity occurs in successive waves, causing a kind of stratification of the neural tube wall, which in this way consists of a number of concentric so-called migration layers. This is in agreement with the opinion of Kodama ('26) who noted that within the ventricular ridge area two different nuclear grisea develop successively both being derivatives of the "same" matrix region. The authors mentioned suggested that all matrix cells display the same generation time, which, however, increases during development. More recently Wächter and Jänsch ('72) noted that as far as the proliferation activity is concerned, the matrix layer in rat embryos would consist of at least two cell populations, the one having a short generation time, the other a long cell cycle.

Conclusively, it can be noted that the matrix layer might display a rather

complicated pattern of intrinsic activity. Possibly, the matrix cells of the originally homogeneous population, exhibit an equal generation time, whereas the different cell types, which originate later, might also show varying cell cycle times.

5.3.3 *The developmental characteristics of the matrix layer*

The investigations of His (1889), Schaper (1897), Spatz ('24) and more recently of Kahle ('51, '56) have contributed to our understanding of the developmental changes within the matrix layer.

Generally, it can be noted that the developmental process of the matrix layer has two aspects: first, the cell production (= proliferation) or intrinsic matrix activity, and secondly, the transfer of cells to the mantle layer, which we will call "the extrinsic matrix activity". Both activities show an increase, a maximum and a decrease. On that account an analysis of the developmental process of the matrix can be described in the following way. At first the matrix consists of a single cell layer, which gradually thickens by a progressive intrinsic matrix activity. Meanwhile more and more cells leave the matrix by an increasing extrinsic matrix activity. After having reached its maximum the intrinsic matrix activity decreases, whereas a high extrinsic activity now causes a gradual shrinkage of the matrix layer. Subsequently, also the extrinsic activity decreases and finally an ependymal layer is left without any mitotic activity.

Kahle ('51, '56) subdivided this developmental process of the matrix into six phases: Three phases of gradual matrix increase, up to a phase of full migration, followed by two phases of gradual exhaustion. However, it must be stressed here that a subdivision in phases of such a dynamic process is a rather heuristic approach. Secondly, it has to be emphasized, that Kahle adopted the premiss that every part of the matrix layer passes through all phases. Nevertheless, this mode of analysis is very profitable, since it results in a clear picture of the matrix development in the various regions of the neural tube.

In his study of the neurogenesis in human embryos, Kahle ('51, '58) provided evidence that matrix development does not occur synchronously in the various regions. This phenomenon, which is called heterochrony, was previously pointed out by Kölliker (1896) and His (1889, '04). The latter pointed out that in the basal parts of the brain the matrix development proceeds faster

than in the dorsal parts. Spatz ('25) noted that caudal regions develop earlier than rostral ones. Both the basodorsal and caudorostral gradients of development were affirmed by Kahle.

In a later paper Kahle ('69) compared the development of telencephalic and diencephalic regions. He asserted that as far as the matrix development is concerned, the medial ventricular ridge is related to the hypothalamic region, whereas the lateral ventricular ridge shows much conformity with the subthalamic region. On that account he suggested that the telencephalic ridges might be rostral extensions of diencephalic regions, as was previously proposed by Grünthal ('52).

From this brief review of the literature it can be concluded that, although the sequential events within the matrix probably are the same, different regions may show a heterochronous development. The latter may be caused by either a different initiation time of the matrix development or by a different rate of that process or both. After having given a description of the method and techniques which we have used, we will study the matrix development within the lateral hemisphere wall including the rostral part of the hypothalamic region against the background of the data from the literature.

5.4 *Method and techniques*

5.4.1 *Method*

In spite of the methodological limitations inherent to subdividing the developmental process of the matrix into a number of phases we essentially used Kahle's ('51, '56) approach. The subdivision in phases was accomplished with the aid of a number of histological criteria both referring to the matrix itself as well as to its relation with the mantle layer and marginal layer. The following criteria have been used:

a. the presence of a cell-free marginal zone;
b. the presence of a mantle layer;
c. the thickness of the matrix layer with regard to that of the adjacent mantle layer;
d. the distinctness of the boundary between the matrix and adjacent mantle layer;
e. the shape of the cells, constituting the matrix and mantle layer;
f. the presence of mitoses.

The combination of these criteria define which phase the matrix shows in a certain region.

complicated pattern of intrinsic activity. Possibly, the matrix cells of the originally homogeneous population, exhibit an equal generation time, whereas the different cell types, which originate later, might also show varying cell cycle times.

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Phase 2: The mantle layer is well developed now, whereas the boundary between it and the matrix layer, although regularly shaped, is less distinct as compared with phase 1. Generally it can be noted that the thickness of both layers is almost equal, and many mitoses can be observed.

Phase 3: In this phase the matrix is in the stage of full migration. It is hardly possible to indicate a boundary between the matrix and the mantle layer. The mantle layer is wider than the matrix.

Phase 4: The first signs of exhaustion of the matrix are indicated by the re-appearance of a distinct, although irregularly shaped border between the matrix and the adjacent mantle layer. However, still quite a number of mitoses are present within the matrix. The mantle layer is now wider than the matrix.

Phase 5: In this phase only a few mitoses, widely spread along the ventricular surface, can be observed in the matrix. The thickness of the mantle layer exceeds by far that of the matrix zone, which now only consists of 3-4 cell layers. The boundary between both layers is distinct and regularly shaped.

Phase 6: The complete exhaustion of the matrix is indicated by the absence of mitoses. The remaining structure is constituted by relatively pale oval to round cells, generally arranged into a single cell layer: the presumptive ependyma.

The subdivision of the developmental process of the matrix is a very artificial one and quite frequently matrices were observed, displaying a character intermediate between phase 1 and 2, or 2 and 3. We have indicated these conditions as subphases 1a and 2a, respectively. In figure 9 the subsequent phases of the matrix development and the code employed in the maps and charts of the matrix-phase regions (figs. 11-20) is shown.

5.4.2 *Techniques*

In each developmental stage the matrix of every tenth or fifth section was analysed and typified according to the phases as defined before. In the drawings of these sections the matrix phases were indicated along the ventricular surface. With the aid of the graphical reconstruction technique the different matrix phase regions were plotted on the median plane. This procedure is schematically shown in figure 10A-B^1-C^1-D^1. In this way the matrix

condition of the various areas in different developmental stages can be visualized. However, it should be emphasized that these figures have a great limitation in obscuring spatial aspects. Therefore, in order to obtain a correct interpretation it is necessary to compare these figures with those given in chapter 4. Secondly, in order to avoid misinterpretations of the matrix condition the direction of sectioning should be perpendicular to the ventricular surface. However, as can be seen in the plates presented in the previous chapter, the shape of the ventricular surface of the lateral hemispheric wall is rather complex. This means that in no direction of sectioning the whole ventricular surface would be cut perpendicularly. In order to minimize the resultant errors, the matrix-phase regions were also indicated on the ventricular surface of the three-dimensional model, which was reconstructed from the same series. In these models those matrix areas which were cut in an unfavourable direction could be pointed out. In these areas the matrices were reanalysed and the interpretation verified by comparison with series of other embryos of a comparable developmental stage, which were sectioned in a more favourable direction. It is evident that only a number of series of embryos of a comparable developmental stage sectioned in different directions can complete the view of the matrix conditions of that particular stage. Another limitation of the technique, which is also related to the complex shape of the ventricular relief is the way in which the observations are visualized. Since, at least in older stages, the projection of the matrix-phase regions on the median plane obscures the relations between these regions and the ventricular sulci. In order to reveal these relations charts have been made in which the relief of the ventricular surface of the lateral telencephalic wall has been transformed into a plane. It has to be emphasized that only in the sections through the foramen of Monro a direct continuity exists between the ventricular surface of the third ventricle and that of the lateral ventricle. This implies that only in those sections distances along the ventricular surface of the lateral ventricle could be measured using the intersection point between the median line and the ventricular surface of the third ventricle as the zero-point.

In all other sections no such continuity exists which makes measurement in that way impossible. For that reason the preparation of the charts mentioned required the introduction of an artificial line of reference. The subsequent steps involved in the preparation of the charts are schematically shown in figure 10 and can be summarized in the following way.

A Phase-typing of the matrix regions as described before.

B 1. Selection of two sections, one through the most rostral part and one through the caudal part of the striatal primordium.
(In the diagram sections C370 and C510).

2. In both of the sections a point (r) on the surface of the ganglionic eminence is marked and the distance of these points to the midline is determined (a and b).

C 1. A system of coordinates is introduced, consisting of a vertical line (M-M), crossed by a number of equidistant horizontal lines. The distance between the latter is determined by multiplying the distance between two consecutively analysed sections by the magnification of the sections.

2. The distances a and b (as derived from the sections C370 and C510, respectively) are plotted on their corresponding horizontal lines of the coordinate system with their zero points coincident with the vertical line (M-M). The two points (r') are marked.

3. The line of reference (R-R) is drawn through these two points.

D All other sections selected are now subjected to the following procedure:

1. In the coordinate system the distance between the line M-M and the line of reference (R-R) is read (i.e. the distance c for section C470).

2. In the drawing of the section a line is introduced parallel to the median line at the distance found (c for section C470). The point of intersection of this line with the ventricular surface of the ganglionic eminence is marked (r'').

3. The distances from this point r'' to the boundaries between the matrix-phase regions and to the sulci are measured on either side along the ventricular surface, using a curvimeter.

E 1. For each section the distances measured are transferred to the corresponding horizontal line of the coordinate system, in which the point of intersection with the line of reference (R-R) is used as indicating the zero point.

2. The charts are completed by connection of corresponding points, both those of comparable boundaries between matrix-phase regions and those belonging to the same sulcus, by drawing best fitting curves.

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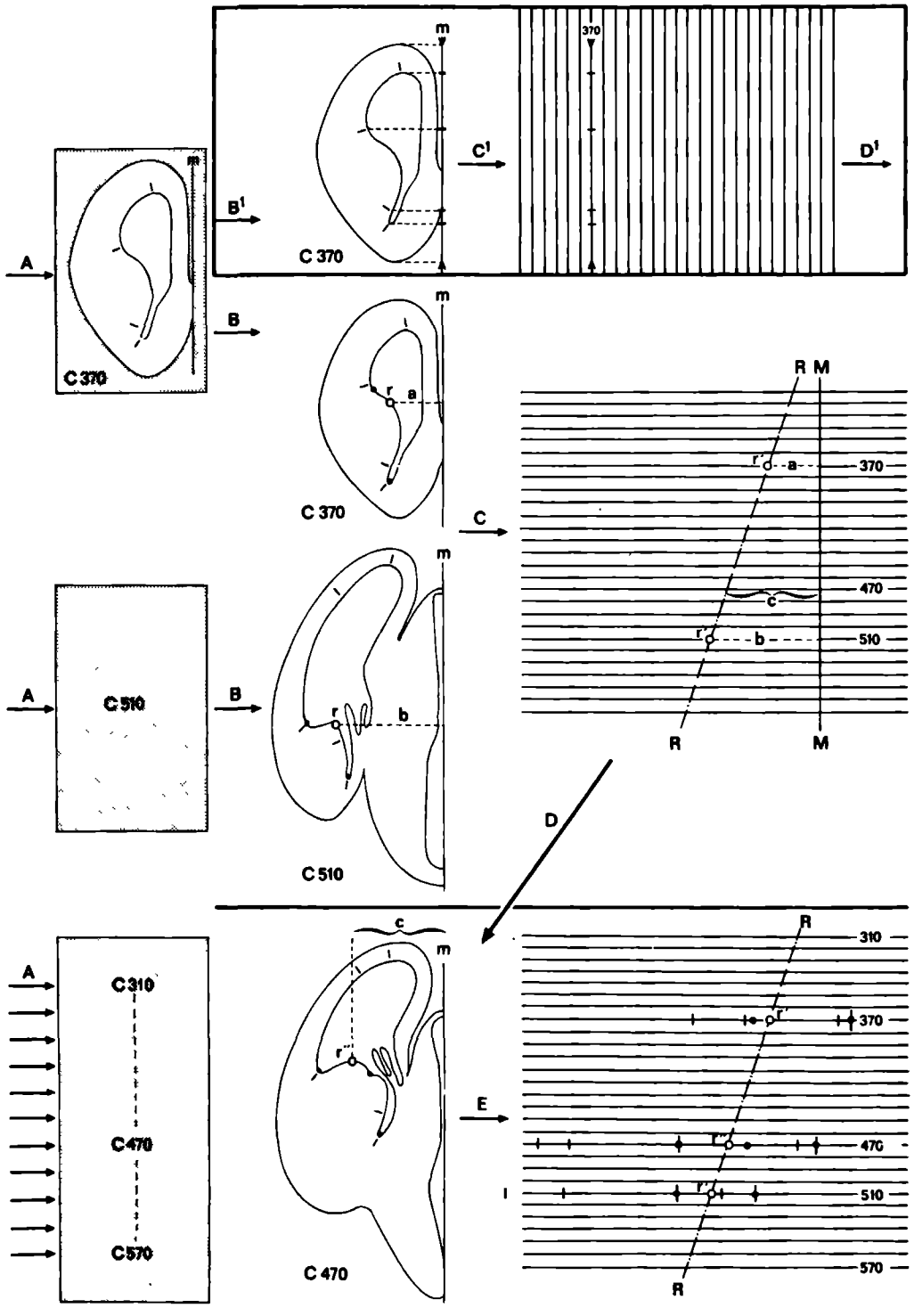
fig. 10 Procedure employed in the preparation of the charts of the matrix-phase regions in the flattened-out lateral hemispheric wall shown in figures 15, 17 and 19. For explanation see text.


A
B
B'
C'
D'
C 370
m
r
a
C
R M
370
c
470
b
510
C 510
D
E
C 470
C310
C470
C570
R
310
570
I

However, it must be emphasized that in this way the transformation only occurs in the direction parallel to the plane of sectioning thus ignoring the curvature of the surface in other directions. This is justified because the aim of these figures is not to visualize the absolute sizes of the different matrix-phase regions but only the relations between these regions on the one hand and the sulci on the other hand.

Using series of embryos of different developmental stages which were sectioned in a similar direction, comparable figures were obtained (figs. 15, 17 and 19).

5.5 *Observations*

Prior to the stage of 12.5 days of development, the matrices of the various areas analysed show a uniform condition. In other words, in this period no boundaries could be observed between regions showing different phases.

The matrix-phase pattern at the stage of 12.5 days

Although the main part of the prosencephalic matrix of this stage still shows a rather uniform condition (fig. 11), a number of areas display a some-

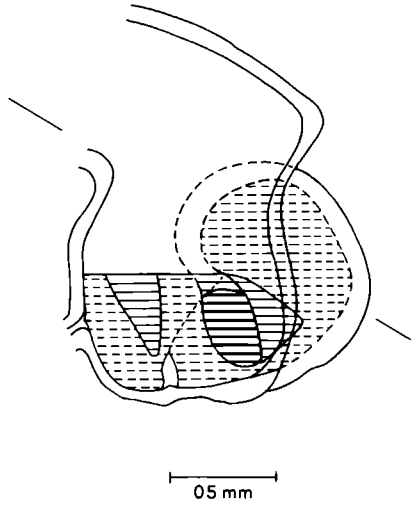


fig. 11 Map of the matrix-phase regions in the lateral hemispheric wall and the rostral part of the diencephalic wall at the stage E12.5 (cf. plate 2).

what advanced character. This holds for a small area roughly corresponding with the dorsal hypothalamic part of the diencephalon showing phase 1, as well as the matrix-region situated at the level of the telodiencephalic transition area. The latter region which corresponds with the medial ventricular ridge (cf. plate 2), shows the condition of phase 1^a. More rostrally and laterally to the latter a region being in phase 1 can be observed, which probably represents the anlage of the lateral ventricular ridge. Thus the matrix pattern at the 12.5 days stage can be described as follows: three somewhat advanced regions exist which are entirely surrounded by areas which still show a matrix condition of phase 0. This pattern suggests the existence of two centres of activity the one being diencephalic, the other at the telodiencephalic border zone.

The matrix-phase pattern at the stage of 13 days

In this stage the two centres of advanced matrix development have both extended and proceeded, but still are surrounded by regions which show no progress at all (fig. 12). The progress of development of the matrix of the

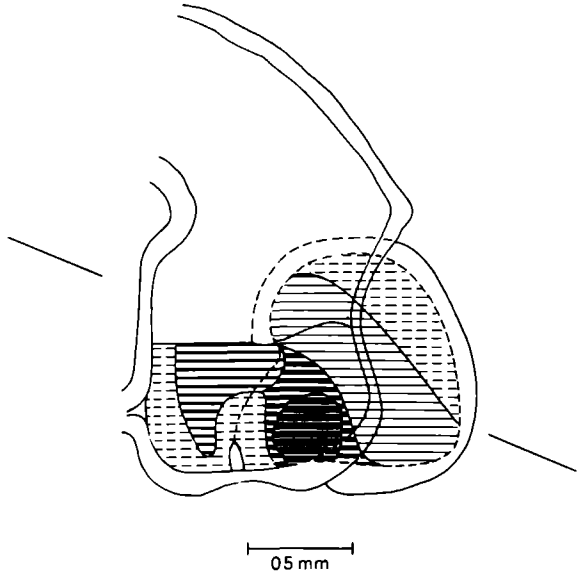


fig. 12 Map of the matrix-phase regions in the lateral hemispheric wall and the rostral part of the diencephalic wall at the stage E13 (cf. plate 3).

medial ventricular ridge is considerable and shows phase 2 and 2^a. This progress is probably related to the rapid expansion of this region during this period of development as is emphasized in the previous chapter (cf. plates 3 and 4).

In the diencephalic region the advanced matrix area has extended and has attained phase 1^a. This area corresponds to the hypothalamic cell-cord of Gilbert ('35). It must be noted that rostrally no continuity exists between the hypothalamic region and the medial ventricular ridge, or between the subthalamic region and the lateral ventricular ridge area, as was suggested by Kahle ('69).

As compared with the previous stage the advances of matrix development can be summarized as follows:

- The area corresponding with the hypothalamic cell-cord of Gilbert ('35) shows a progressive condition.
- The matrix of the medial ventricular ridge is by far the most advanced area of the entire prosencephalon.

The matrix-phase pattern at the stage of 13.5 days

The matrix-phase regions are visualized in figure 13. The most remarkable change in respect to the former stage is that two areas have entered phase 3, being the phase of full migration. At the diencephalic level it is the area which mainly corresponds with the hypothalamic cell-cord. More rostrally the matrix of the main part of the medial ventricular ridge has also attained this phase. It is noteworthy that the most basal area of the hypothalamus is constituted by a narrow elongated region in which the matrix development is retarded and still shows the condition of phase 0. It should also be noted that the preoptic region, which forms part of the medial ventricular ridge, is retarded in respect to the remainder of this ridge. Rostrally and laterally to the medial ventricular ridge a somewhat advanced matrix area is situated, which roughly corresponds with the lateral ventricular ridge. Dorsally to this area an elongated, relatively wide area is located, showing phase 1; still more dorsally, the pallial region, showing phase 0, is located. Thus, the overall picture of matrix-phase regions within the lateral hemisphere wall displays an organisation in columnar areas extending in a caudorostral direction.

In comparison with the former stage the progress of matrix development can be summarized as follows:

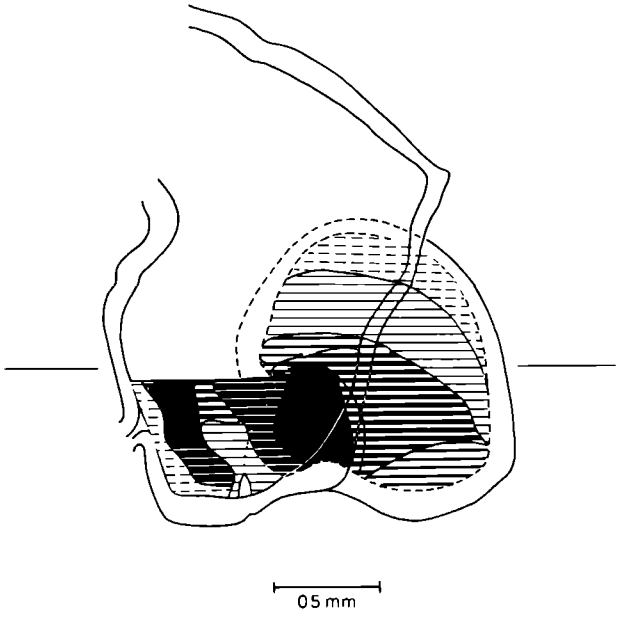


fig. 13 Map of the matrix-phase regions in the lateral hemispheric wall and the rostral part of the diencephalic wall at the stage E13.5 (cf. plates 6 and 7).

- The hypothalamic cell cord and the medial ventricular ridge have attained phase 3.
- Within the most basal hypothalamic region an elongated, retarded area remains.
- The preoptic region lags behind with respect to the rest of the medial ventricular ridge.
- The lateral ventricular ridge shows some progress although it is still retarded as compared with the medial ventricular ridge.

The matrix-phase pattern at the stage of 14 days

As shown in figure 14, retarded as well as progressive areas can be observed within both the hypothalamic region and the lateral telencephalic wall. The difference, however, is that within the hypothalamus the retarded region is located basally, whereas in the telencephalon these regions are situated dorsally.

Within the hypothalamus the most advanced area has already reached the phase of beginning exhaustion (phase 4). The region dorsal to the hypothalamic

medial ventricular ridge is considerable and shows phase 2 and 2^a. This progress is probably related to the rapid expansion of this region during this period of development as is emphasized in the previous chapter (cf. plates 3 and 4).

In the diencephalic region the advanced matrix area has extended and has attained phase 1^a. This area corresponds to the hypothalamic cell-cord of Gilbert ('35). It must be noted that rostrally no continuity exists between the hypothalamic region and the medial ventricular ridge, or between the subthalamic region and the lateral ventricular ridge area, as was suggested by Kahle ('69).

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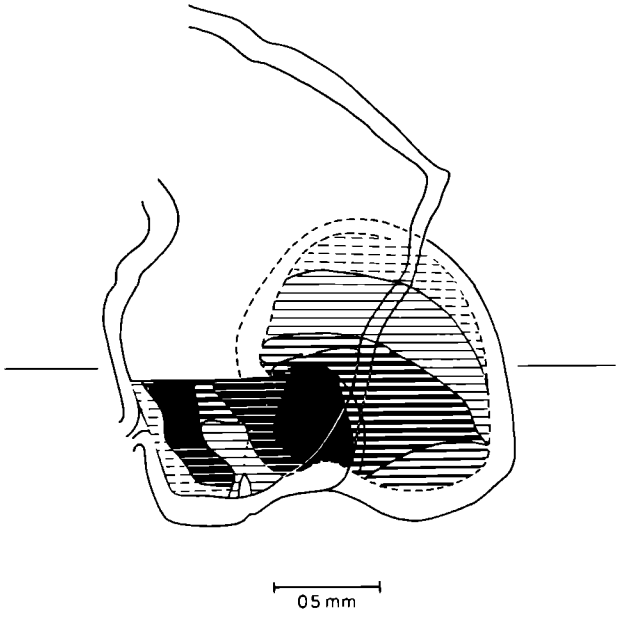

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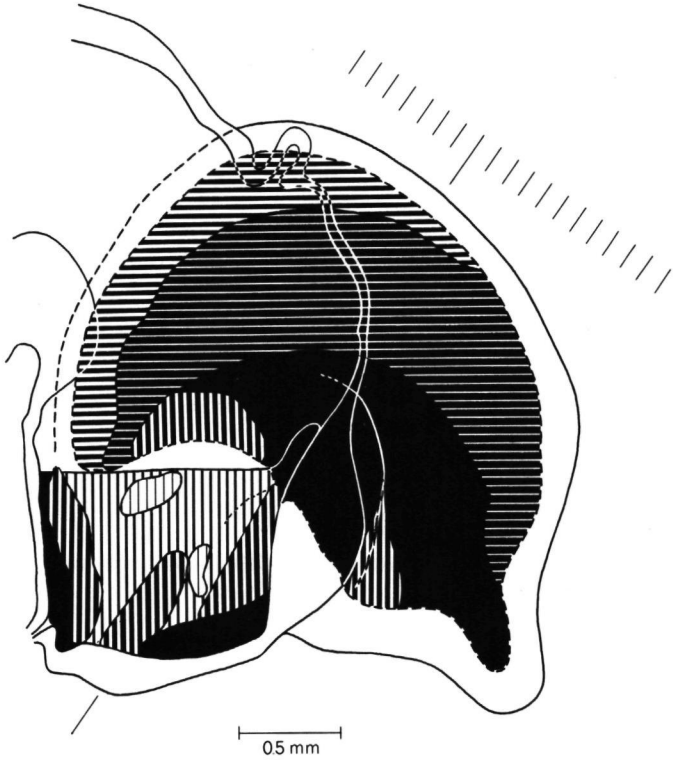

fig. 16 Map of the matrix-phase regions in the lateral hemispheric wall and the rostral part of the diencephalic wall at the stage E16 (cf. plates 13-16).

The condition of the matrix of the hypothalamic region is far more progressive than that found in the telencephalic regions. Here the matrix of the basal part, corresponding with the entire ventricular elevation, mainly displays the phase of full migration (phase 3). As compared with the former stage it must be noted that the matrix of the lateral ventricular ridge has now reached the same phase, i.e. phase 3 as the medial part. It can also be seen that within the elevated area two regions have entered the phase of beginning exhaustion: rostrally the area on both sides of the sulcus subpallii intermedius and caudally a medial area of the undivided part of the ventricular elevation. Also in this stage the condition of the dorsal pallial region remains retarded. Figure 17 displays the chart of the flattened-out surface of the lateral te-

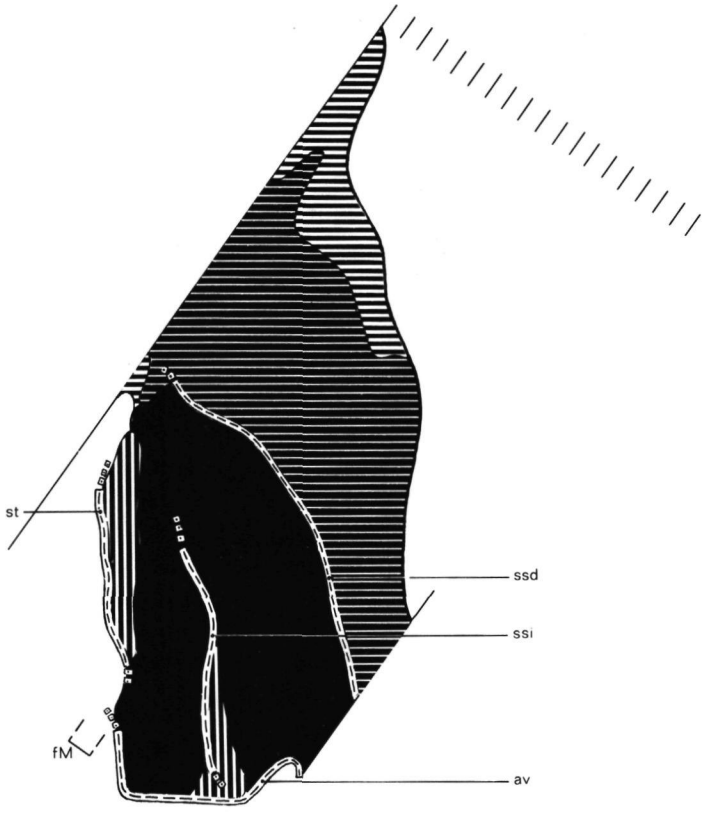

fig. 17 Chart of the matrix-phase regions in the flattened-out lateral hemispheric wall at the stage E16 (cf. fig. 16).

lencephalic wall. It is shown that the sulcus subpallii dorsalis (roughly) coincides with a boundary between two matrix phase regions.

The progress of matrix development can be summarized as follows:

- The hypothalamus is considerably advanced as compared to the lateral telencephalic wall.
- The main part of the preoptic region displays the same pattern as the basal telencephalic region.
- The medial ventricular ridge still shows a condition of full migration.
- The lateral part of the ventricular eminence has now reached the same phase as the medial part (phase 3).
- Two parts of the elevated area have entered the phase of beginning exhaustion.

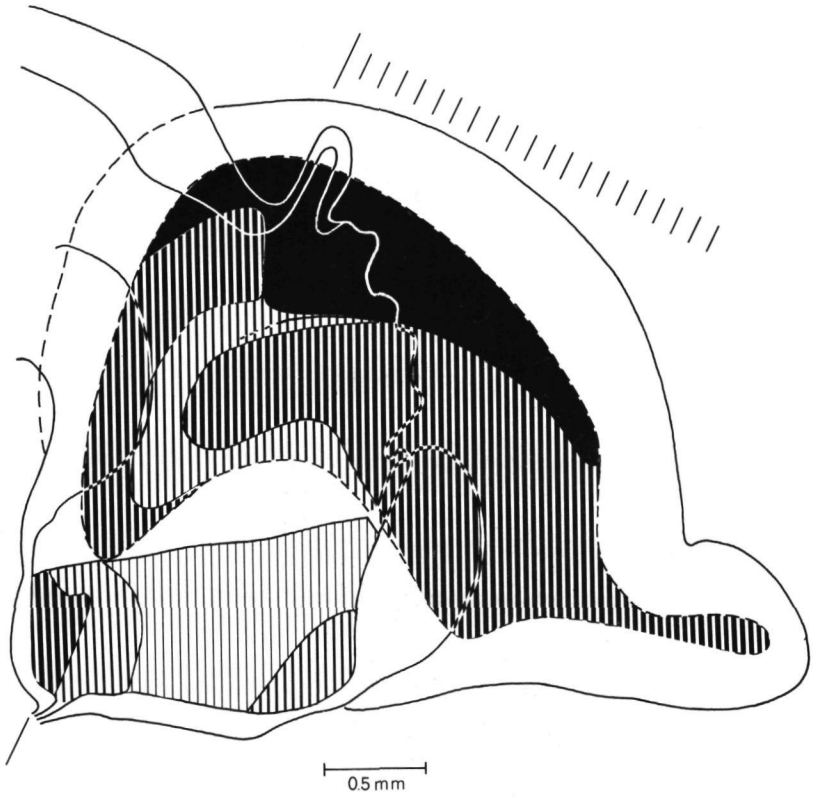

fig. 18 Map of the matrix-phase regions in the lateral hemispheric wall and the rostral part of the diencephalic wall at the stage E18 (cf. plate 17).

The matrix-phase pattern at the stage of 18 days

Figure 18 shows the matrix phase regions at the 18 days stage. The tendency of development, already shown in the 16 days stage has continued. In the hypothalamic region the main part of the matrix has reached the final stage of exhaustion and is transformed into an ependyma-like structure, without any mitotic activity (phase 6). Only a part of the preoptic region plus the basal hypothalamic region are somewhat retarded. Within the lateral telencephalic region, however, no part of the matrix has reached the final stage of exhaustion as yet. Only a relatively small caudal part of the ventricular eminence, having two rostral extensions along the sulcus terminalis and the sulcus subpallii dorsalis, shows phase 5 of advanced exhaustion, the rest of the ventricular eminence is in the phase of beginning exhaustion (phase 4).

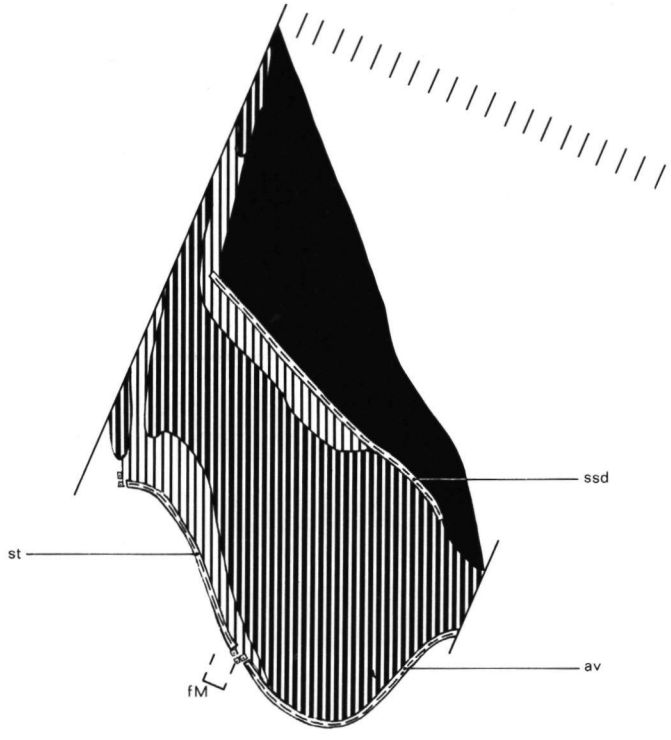

fig. 19 Chart of the matrix-phase regions in the flattened-out lateral hemispheric wall at the stage E18 (cf. fig. 18).

A striking feature is that the matrix of the area corresponding with the presumptive pallium, although also still retarded, now has reached the phase of full migration (phase 3). The pattern of matrix development at this stage is also visualized in the flattened way (fig. 19). It shows very clearly the almost uniform matrix condition of the ventricular eminence bordered by the more advanced regions (phase 5) along the two sulci, whereas the pallial region is retarded.

The advances in the matrix development as compared with the previous stage can be summarized as follows:

- The main part of the hypothalamic region displays an exhausted matrix, with the exception of the basal part.
- The most rostral part of the preoptic region lags behind as compared to the hypothalamus.

- Almost the entire ventricular eminence displays a beginning exhaustion.
- The pallial region has entered the phase of full migration.
- The diencephalic region analysed is clearly advanced as compared to the lateral telencephalic wall.

5.6 *Discussion and conclusions*

As was noted before, the developmental process of the matrix of the neural tube shows both a caudorostral gradient (Spatz, '27) and a baso-dorsal gradient (His, '04), as was affirmed by Kahle ('51, '56). Our observations are in agreement with these views although it should be emphasized that this pattern is not a rigid one. The caudo-rostral gradient is clearly shown in figure 18: at the stage of 18 days the matrix of the rostral diencephalon is already entirely exhausted, whereas the matrix of the telencephalic regions lags behind. The baso-dorsal gradient is clearly present in the telencephalon wall, but in the hypothalamic region a basal longitudinal area remains retarded as compared with the dorsally situated hypothalamic cell cord, thus contradictory to the general concept. Keyser ('72) arrived at a similar conclusion.

Another striking feature is that the developmental process of the prosencephalic matrix appears to be initiated at two centres (fig. 11 and 12). The one roughly corresponds with the hypothalamic cell cord, the other is situated at the level of the medial ventricular ridge. Subsequently, the developmental process seems to spread from these centres to the surrounding areas, although this occurs in different ways. In the analysed part of the diencephalon no special programme can be discerned, whereas the telencephalic regions seem to be organized in longitudinally oriented columns. This columnar pattern would be in agreement with a similar organization of the mantle layer, as described by Holmgren ('25) and Källén ('51).

We will now turn to a comparative analysis of the developmental process of the matrix in the following four regions: the preoptic region, the medial ventricular ridge, the lateral ventricular ridge and the pallial region. The results of this analysis are schematically shown in figure 20. However, it has to be noted that each region not always displays one and the same matrix condition throughout its extent. In those cases that matrix phase having the greatest extension was considered to be determinant.

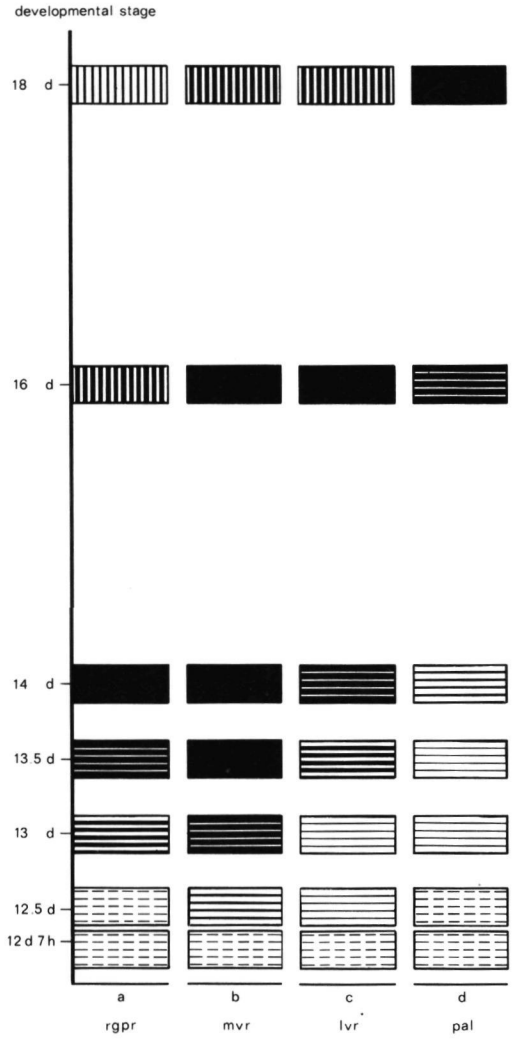

fig. 20 Diagram showing the development of the neuroepithelium in various prosencephalic regions (cf. fig. 9):
a. preoptic region
b. medial ventricular ridge area
c. lateral ventricular ridge area
d. pallial region

As was noted in the previous chapter, the preoptic region and the ventricular ridge together constitute a single morphogenetic entity. In the figures 20a and 20b the matrix development of these regions is indicated. It is clearly shown, that in the medial ventricular ridge the process starts somewhat earlier, and enters the phase of full migration at 13.5 days. The preoptic region lags behind up to the stage of 14 days when it also displays phase 3. During later development this relation becomes reversed, since the medial ventricular ridge retains phase 3 during the whole period between 13.5 and 16 days, whereas the preoptic region only shows phase 3 at 14 days and after that time rapidly gets exhausted. From these results it can be concluded that, unlike the morphological development, the matrix of the preoptic region develops rather independent from that of the medial ventricular ridge.

In a comparison of the morphogenesis of the two ventricular ridges evidence was provided in the previous chapter that the medial ridge appears at 12.5 days, whereas at 13 days the first indication of the lateral one can be discerned. As shown in the figures 20b and 20c the developmental process of the matrix of both ridges is initiated at 12.5 days. However, it is also obvious that prior to the 16 days stage the progressive tendency of both ridges is quite different. The medial ventricular ridge enters the phase of full migration i.e. phase 3 rather quickly at 13.5 days and retains that phase up to the 16 days stage, whereas the lateral ventricular ridge only attains this phase at 16 days. This might, in part at least explain the morphogenetical difference between both ridges. After the 16 days stage both ridges show an equal progress of matrix development. The latter synchronization coincides with the disappearance of the sulcus subpallii intermedius. In this way a morphologically single ventricular eminence is constituted, which also shows an almost uniform matrix condition.

The differences in matrix development of the two ridges has been pointed out already by Kodama ('26) and Kahle ('69). Both authors supposed that during the intermediate developmental period, in which no sulcus exists in the caudal part of the ventricular eminence, a different matrix condition would indicate a border between the two original parts. However, our observations do not support this view, since during the period between 13.5 and 16 days the caudal part of the eminence shows a uniform matrix condition. Nor is there any evidence in favour of the hypothesis that the caudal part might originate independently from both ridges, gradually pushing the rostral, divided part forwards, as was alternatively proposed in the previous chapter. The fact that

the matrix of the caudal part develops similar to that of the lateral ventricular ridge suggests that this part might only be derived from the lateral ridge. However, it has to be emphasized that the sulcus subpallii intermedius, if present, usually does not coincide with a borderline between matrix phase regions, although it indicates the morphological border between the two ridges. On that account we agree with the view of Källén ('51), Källén and Bergquist ('54) and Keyser ('72) who stated that ventricular sulcii must be interpreted as morphological structures which originate secondarily to histogenetical events. In our opinion the undivided caudal part of the ventricular eminence originates by union of both ridges.

In figure 20d the matrix development of the pallial region is indicated. It can be seen that this region is the most retarded area of the lateral telencephalic wall. The pallial region enters the phase of full migration rather late in development, namely at 18 days. This means that only then the time is reached in which much cellular material is transferred from the matrix layer to the mantle layer and an increase in thickness of the wall occurs. This, partly at least, explains the proportional morphological changes appearing in this phase of development, as described in the previous chapter. Our observations (fig. 20c and d) do not support the view of Ariëns Kappers ('23) who suggested that the lateral ventricular ridge is in part of pallial origin. Moreover, it should be emphasized that one can only properly indicate a pallial region when the subpallial area is present. Finally, it should be noted that our results do not entirely correspond with those of Grünthal ('52) and Kahle ('56, '69). These authors suggested that in early development the matrix of the hypothalamus progresses similar to that of the basal area of the telencephalon. On this account they considered the hypothalamus and the basal telencephalic region as constituting a single histogenetic entity. Although both areas seem to be centres of early matrix activity, the differences in the development of their matrices in our opinion do not support such a conclusion.

6 THE MANTLE LAYER

6.1 *Introduction*

After the development of the matrix layer, as described in the preceding chapter, the formation of the mantle layer is the second step in the histogenesis of the central nervous system. In the development of the neuronal constituents of the developing mantle layer three main aspects can be distinguished. First, the formation of the neuroblasts, which occurs either in the matrix or in the submaternal layer. Secondly, the migration of the neuroblasts to their destination. Finally, the differentiation of the neuroblasts into the mature neurons, which ultimately constitute the various nuclei and the development of the fibre connections of the latter.

In the ensuing chapter first a brief survey will be given of the literature concerning these three aspects. Subsequently, attention will be paid to the origin and differentiation of the various nuclei constituting the strio-amygdaloid complex and to the development of some related fibre connections. An analysis of the time of origin and the migration of the neuroblasts in the strio-amygdaloid complex will be presented in the next chapter.

6.2 *Literature*

6.2.1 *Formation of neuroblasts*

In early embryonic stages the neuroepithelium gradually develops into a pseudostratified epithelium representing the matrix layer. Its constituent cells are attached both to the internal and external limiting membranes by cytoplasmic processes. At the ventricular surface the inner process is anchored firmly to the adjacent cells by terminal bars (Langman et al., '66, Langman, '68). After their final mitotic division the cells, which may now be called young neuroblasts, loose their contact with the internal limiting membrane and migrate towards the mantle layer. The mechanism by which the neuroblasts become detached is still a matter of conjecture (Hamburger and Levi-Montalcini, '50; Langman et al. '66; Langman, '68). The young neuroblasts are distinct from the matrix cells in having a large round nucleus with a pale nucleoplasm and a darkly stained nucleolus. Besides the neuroblasts have lost the ability to synthetize DNA for a next mitotic division (Langman et al. '66). However, in later stages a second layer arises in the telencephalon, which probably also gives rise to neuroblasts: the submaternal layer in our

terminology. As early as 1897 Schaper noticed extra-ventricular mitoses occurring peripherally to the matrix in some regions where the matrix layer had attained a considerable thickness. Hamilton ('01) and Allen ('12) affirmed Schaper's findings and Allen noted the persistence of a mitotically active layer bordering the lateral ventricles in developing rat brains up to two years after birth.

Opalski ('34) provided evidence for the existence of such a layer in man; he considered it as a remnant of the matrix layer. Globus and Kuhlenbeck ('44) also observed the layer in question in human material. They coined the term "subependymal plate" for this structure and suggested that it might be a source of cerebral gliomas. Altmann ('62, '63) autoradiographically demonstrated that even in adult rat brains new cells are formed, without traumatic tissue damage. He suggested that these new cells probably originate from the subependymal plate since this structure shows a number of mitotic figures as also indicated by Fleischhauer ('61) and Kirsche ('67). On that account the latter authors called it postembryonal matrix.

Recently Fleishhauer ('72) reviewed the literature concerning the subependymal plate. He emphasized that its existence is variable in different species and he defined this structure as a meshwork of glia fibres followed by a row of astrocytes underneath the ependyma of most regions of the cerebral ventricles. In other words it has to be considered as a structure belonging to the adult brain. At this juncture it is necessary to stress the fact that the term subependymal layer is not always used in this way. The subependymal plate in adults should be distinguished from the submaternal layer in our terminology which is an embryonic structure situated external to the matrix (and in later development to the ependyma) and which probably persists up to the adult stage as the subependymal plate. In this way our submaternal layer corresponds to the "Keimlager" of Kahle ('51, '69), to the subependymal layer of many authors (among them Smart, '61, '72, '73; Stensaas and Gilson, '72; Privat and Leblond, '73; Paterson et al. '72); and to the subventricular zone of the Boulder Committee ('70).

With regard to the development of the submaternal layer it should be noted that, in intermediate developmental stages, peripheral to the matrix layer a structure appears, which is constituted by a great number of indifferent, mitotically active, cells. The layer is very prominent in the ventricular ridge area. Kahle ('69) noted that in contradistinction to the matrix layer the submaternal layer is less important to the cell production, but

rather had to be considered as a stock of indifferent cells which gradually migrate towards the mantle layer. This is in agreement with the opinion of Kodama ('26). Furthermore Kahle mentioned the difference in time of origin and ultimate fate of the matrix, and the submaternal layer: the appearance of the submaternal layer occurs in a later period of development than the matrix layer, moreover the submaternal layer persists after the matrix layer is exhausted and the ependyma is formed. The transformation of the submaternal layer into the subependymal plate occurs gradually and is still going on when the ependymal layer already covers the surface of the lateral ventricle. Studying the submaternal layer only at late developmental stages as has been done by several investigators (Smart and Leblond, '61; Noetzel and Roux, '64; Westergard, '70) it does occupy a subependymal position, yet it should be considered still as the submaternal layer, which for the sake of clarity has to be distinguished from the subependymal plate proper which is present in the adult stage. Moreover it is misleading to consider the subependymal plate of the adult as a remnant of the matrix layer, as has been suggested by some of the authors mentioned above.

It is suggested that the submaternal layer plays a role in the production of both neurons and glial cells. A number of authors among them Smart and Leblond ('61), Noetzel and Roux ('64) have expressed the opinion that the constituent elements of this layer can be subdivided morphologically into two groups, possibly representing neuronal and glial precursors, respectively. However, Stensaas and Gilson ('72), studying the caudatopallial junction area in the lateral ventricle of the neonatal rabbit, rejected this idea since the primitive submaternal cells have no features which would clearly relate them to nerve or glial cells. These authors stated that all of the cell elements of this layer have to be interpreted as transitional forms within a single homogeneous population of mitotically active cells. Nevertheless, it has to be noted that the submaternal layer, at least in intermediate developmental stages, plays a role in the neuroblast production.

6.2.2 *Migration of neuroblasts*

After the neuroblasts have entered the mantle layer they migrate towards their ultimate position. With regard to this migration process some different opinions exist.

Especially some earlier neuroembryologists believed that the migration occurs as an amoeboid movement of the entire cells (Schaper, 1897). More

recently Levi-Montalcini ('64) agreed with this opinion. She noted that cells which are preparing to migrate become spindle-shaped. However, this author stresses the fact that the migration process in the central nervous system is not a chaotic one, but on the contrary is highly organized and rigidly patterned. Several later investigators have rejected the possibility of an amoeboid movement of the cells. Thus, Berry and Rogers ('65) and Morest ('70) suggested a migration movement of the nucleus within its own cytoplasmic processes which are attached to both the inner and outer surface of the brain wall. Rakic ('72), on the other hand provided evidence that radially arranged glial fibres serve as guidelines for the movement of neuroblasts. However, it should be emphasized that in some brain regions (pons, cerebellum) cells migrate parallel to the external surface. It is not known whether also in this so-called tangential migration a fibre system provides a guiding mechanism for the migration of the neuroblasts.

6.2.3 *Differentiation of neuroblasts*

After having reached their ultimate position within the mantle layer the neuroblasts differentiate into mature neurons by the formation of axons, dendrites and synaptic connections. However, it should be emphasized that the distinction between neuroblasts and neurons is very difficult and depends on the technique used (Ramon y Cajal, '09; Hamburger and Levi-Montalcini, '50; Morest, '69a, b, '70).

Using the Golgi technique Morest provided evidence that the differentiation of the postmigratory neuroblast is initially marked by the outgrowth of its axon. Subsequently the dendrites are formed and the synaptic connections are established. Finally, the myelination of the axon completes the differentiation process. Morest supposed that the afferent axons might induce or at least influence the differentiation of the neuroblasts, as was also suggested by Levi-Montalcini ('49). Whether the inducing mechanism might be chemotaxis or physical contact remains to be elucidated. The order in which the subsequent events of the differentiation occur is probably similar in all regions, although the timing of those events is regionally patterned. Generally it can be noted that large neurons differentiate earlier than small ones.

No unanimity exists in the literature concerning the way in which the various grisea originate. According to Herrick ('33) the nuclei are formed by differentiation centres represented by regions of the matrix layer, which are bounded by ventricular sulci. Bergquist and Källén ('54), on the contrary, held that during development sulci mark the centres rather than the boundaries

of the areas from which the various grisea develop. Rose ('42) believed that the matrix gives rise to transitional "pronuclei"; the definitive nuclei would originate either by subdivision or by fusion of these pronuclei.

6.2.4 *The nuclei constituting the strio-amygdaloid complex*

The development of the brain nuclei (or grisea) within the subpallial region, has been studied by a large number of investigators in a variety of species. Ziehen ('06; some mammals); Hochstetter ('19: Homo); Johnston ('23: Homo); Ariëns Kappers ('23; chick and Homo); Spatz ('24, '25: Homo); Kuhlenbeck ('24, '26: various submammalian and mammalian vertebrates); Holmgren ('25: mus musculus); Kodama ('26: Homo); Cooper ('46: Homo); Macchi ('51: Homo); Källén ('51a: some vertebrates and Homo); Grünthal ('52: mouse); Humphrey ('67, 72: Homo); Kahle ('69: Homo). As regards the development of the globus pallidus the work of Richter ('65, '66) should be especially mentioned.

Before giving a description of the observations concerning the mantle layer development within the subpallial region in the Chinese hamster, some general remarks should be made. The nuclei which we have studied are those belonging to the so-called strio-amygdaloid complex.

We also studied the differentiation of some fibre tracts, namely the stria terminalis and the commissura anterior. These tracts appear rather early in development and show a close relation to some of the nuclei of the strio-amygdaloid complex. This complex can be subdivided into:

1. the corpus striatum, comprising:
   - the nucleus caudatus and the putamen: which in the adult Chinese hamster form a single homogeneous griseum.
   - the globus pallidus

2. the nucleus amygdalae, composed by:
   a) a basolateral cellgroup containing:
      - the nucleus basalis amygdalae
      - the nucleus lateralis amygdalae
   b) a cortico-medial cellgroup containing:
      - the nucleus corticalis amygdalae
      - the nucleus medialis amygdalae
      - the nucleus centralis amygdalae

c) a so called rest group containing:
   - the nucleus tractus olfactorius lateralis
   - the area amygdaloidea anterior
   - the massa intercalata

The two cell masses last mentioned consist of diffusely arranged cells. They can hardly be demarcated from the surrounding grisea. Hence they have been omitted in the pictures accompanying this chapter.

The subdivision of the amygdaloid complex presented above is based upon the topographic position of the grisea in adults. Recently Lammers ('72) stressed the fact that some of the nuclei can hardly be considered as belonging to the amygdaloid complex when their hodological relationships are taken into account.

In the study of the development of the mantle layer we employed series stained according to the haematoxilin and eosin and Nissl technique. It is important to know that these stainings show hardly more than the nuclei of the cells. For that reason the delimitation of the (primordial) grisea relevant to this study was carried out on the basis of the following criteria:

a. the density of the cell nuclei
b. the presence of areas devoid of cells, bordering the grisea
c. the characteristic size and shape of the cell nuclei, constituting a certain griseum
d. the presence of fibres between the cell nuclei.

For the study of the development of the fibre connections within the subpallial region we made use of series which were stained according to the Bodian as well as the Palmgren technique.

In studying the adult brain we employed the staining techniques according to Klüver-Barrera, Nissl and Bodian. As guides for the delimitation of the brain nuclei in adults the atlasses of König and Klippel ('63: rat); Luparello ('67: cavia) and Knigge and Joseph ('68: golden hamster) were used.

As in the former chapters we also studied the mantle layer development by employing a series of closely graded stages of development. In order to visualize the results graphical reconstructions were made, showing the position of the mantle layer and/or its derivatives as viewed from the medial aspect.

The stages of 12,5 and 13 days

Morphologically the stage of 12,5 days is characterized by the appearance of the medial ventricular ridge (plate 2), whereas at the 13 days stage the first indication of the lateral ventricular ridge is present (plates 3 and 4). A mantle layer, consisting of round, pale, scattered cells, can first be discerned at 12,5 days at the level of the medial ventricular ridge. At the 13 days stage a mantle layer is also present in the lateral ventricular ridge area.

In neither of the two stages mentioned any differentiation could be observed and thus no primordial grisea could be delimited. The only remarkable feature is that some extraventricular mitoses can be observed in the medial ventricular ridge area at the junction between the matrix and the mantle layer. Although a clear submaternal layer is not yet present, these mitoses indicate the position where this layer will manifest itself in later stages.

The stages of 13,5 and 14 days

Morphologically the stage of 13,5 days clearly shows the presence of the medial as well as the lateral ventricular ridge, whereas caudally both ridges have started to unite (plates 6, 7 and 8).

As regards the mantle layer development this stage displays a considerable progression when compared with the former stage, since the first primordial grisea can be discerned. This holds in particular for the subthalamic and hypothalamic regions and for the medial ventricular ridge area. Within the latter area a clear submaternal layer is present, as has been indicated in plate 20. However, in the centre of this region no sharp boundary can be indicated between the submaternal layer and the matrix layer, which suggests a strong participation of the latter in the development of the former. A great number of mitoses can be observed within the submaternal layer.

In the caudal part of the medial ventricular ridge lateral to the submaternal layer a well individualized cell group can be delimited within the mantle layer (plate 20). This cell group can be recognized as the primordial globus pallidus because it consists of loosely arranged pale cells which are intermingled with fibre bundles. The nuclei of these cells are spindle shaped with their greatest diameter directed parallel to the fibres. These fibres seem to converge towards a massive fibre bundle which caudally extends to the

presumptive sub- and hypothalamic region respectively. This bundle was termed the "stem bundle" by His ('04). The spatial relationships of both the primordial globus pallidus and the stem bundle are visualized in plate 21.

The stage of 14 days shows a strong intraventricular expansion of both ventricular ridges (plates 10 and 11). Now a clear submaternal layer is also present in the lateral ventricular ridge area. Within the diencephalon the further differentiation of the mantle layer results in the appearance of a great number of pronuclei and fibre tracts (see Keyser '72). However, within the subpallial region no further segregation into primordial grisea can be recognized. Summarizing it can be noted that both the globus pallidus and the stem bundle have extended when compared with the 13,5 days stage.

The stage of 15 days

The mantle layer development of the subpallial area in this stage is characterized by the incipient differentiation of the caudatus-putamen complex, which is situated mainly dorsally and rostrally to the globus pallidus (plate 22). It should be noted that only the lateral boundary of this complex can be indicated, since there a cell-free zone is present. Rostrally the caudatus-putamen complex borders on the submaternal layer of the lateral ventricular ridge, whereas medially it is bounded by the submaternal layer of the medial ventricular ridge.

As regards the differentiation within the strio-amygdaloid complex, only a single condensation of cells could be noted, which probably corresponds with the primordial nucleus centralis amygdalae. As compared with the former stages the advancement in differentiation of the mantle layer can be summarized as follows:

- the development of the primordial putamen-caudatus complex.
- the appearance of the presumptive nucleus centralis amygdalae.

The stage of 16 days

In the 16 days stage the ventricular ridge area is still subdivided into two parts (plates 13, 14, 15 and 16). As regards the mantle layer differentiation it should be noted that this stage shows quite a progression when compared with the preceding stage.

The caudatus-putamen complex is well differentiated (plate 23a, b) although its caudal border is rather ill defined. As in the former stage, a close relation exists between this complex and the submaternal layer of both

ventricular ridge areas. The caudatus-putamen complex is situated mainly rostrally and laterally to the globus pallidus. Histologically the complex is constituted by a rather uniform mass of cells, which, particularly caudally, is invaded by fibres of the developing capsula interna. The latter phenomenon holds also for the globus pallidus, which shows a conspicuous differentiation.

Within the presumptive amygdaloid region the corticomedial group can now be identified rather well. This holds in particular for the nucleus centralis amygdalae, situated caudally to the globus pallidus and medially to the caudal part of the caudatus-putamen complex. A close topographic relation exists between the nucleus centralis and the massa cellularis reuniens superior on the one hand and the stria terminalis on the other (plate 24). The anlage of the nucleus corticalis and the nucleus medialis amygdalae can also be discerned, although their delimitation is rather difficult (plate 23c). The basolateral group presents itself as a rather undifferentiated accumulation of cells, which could not be subdivided. Grisea belonging to the restgroup of the amygdaloid complex could not be identified. The spatial relationships of the various identified nuclei are visualized in plate 24, in which the stria terminalis and the commissura anterior are also indicated.

The stria terminalis passes as a slightly curved bundle from the primordial amygdaloid complex to the preoptic region. Some of the stria terminalis fibres cross the median plane via the anterior commissure, thus forming the commissural component of the stria terminalis. The rest of its fibres diverge rostrally. Within the torus transversus the commissura anterior can be recognized, which in this stage only shows an anterior limb. Finally it should be mentioned, that a great number of fibres which seems to originate from the, still poorly differentiated, dorsal thalamic region, run rostrally towards the cerebral stem area (plate 23b). There they curve laterally passing ventrally to the stria terminalis just caudal to the foramen of Monro. Thence, these fibres reach the striatum, while some of them can be traced laterally and dorsally into the presumptive cortex region. These fibres constitute together the anlage of the capsula interna. The progressive diminution of the foramen of Monro occurring in this stage can be related to the massive development of the internal capsule.

Plate 24 shows that the cell masses of the strio-amygdaloid complex as a whole is slightly curved. During further development this situation will change considerably.

Summarizing it may be stated that the stage of 16 days is a crucial one since most cell groups belonging to the strio-amygdaloid complex can now be recognized. All of the nuclei of the corticomedial group (i.e. the nucleus centralis, medialis and corticalis) can be delimited, whereas within the basolateral group no clear subdivision is possible as yet. In addition some important fibre tracts, namely the stria terminalis, the commissura anterior and the capsula interna can be recognized.

The stage of 18 days

The morphological characteristics of the 18 days stage are the complete fusion of both ventricular ridges and the advanced formation of the cornu inferius ventriculi lateralis (plate 16).

The matrix of the subpallial region shows a beginning or even advanced exhaustion (c.f. fig. 18), and a wide submaternal layer containing a great number of mitotic figures can be discerned in this area (plate 25).

As can be seen in plate 26 the further differentiation within the mantle layer has mainly resulted in an considerable outgrowth of the various grisea. This holds in particular for the caudatus-putamen complex, which shows an enormous dorsoventral expansion. Rostrally a close relationship exists between this complex and the submaternal layer, but more caudally the two structures mentioned are separated by fibres of the capsula interna (plate 25). The globus pallidus has now acquired a more ventral position with regard to the caudatus-putamen complex.

The corticomedial group of the amygdaloid complex shows a further expansion of its three constituent nuclei. Within the basolateral group the nucleus basalis and lateralis amygdalae are now almost entirely separated. The direct relationship of this group with the ventricular wall of the cornu inferius can be deduced from plate 26. Within the so called rest group of the amygdaloid complex the primordial nucleus of the tractus olfactorius lateralis can be discerned.

Concerning the fibre tracts of the subpallial region it should be mentioned that at this stage in many places small dark cell nuclei can be observed within the various fibre bundles. Possibly these cells might be derived from the adjacent submaternal layer. The curvature of the stria terminalis has increased and the same holds for the strio-amygdaloid complex as a whole (plate 26). Both phenomena as well as the formation of the cornu inferius are probably related to the expansion of the cerebral stem area. The

expansion of the latter is in its turn related to the outgrowth of the internal capsule.

In the 18 days stage the posterior limb of the anterior commissure appears. This limb is situated basally to the caudatus-putamen complex, as can be seen in plates 26 and 25b. As was mentioned in the previous chapter the neocortex starts to differentiate at 18 days, and this differentiation is accompanied by the incipient development of the corpus callosum.

Summarizing, it may be stated that the following structures can first be recognized in the 18 days stage:

- the nucleus basalis amygdalae and the nucleus lateralis amygdalae;
- within the rest group the nucleus tractus olfactorius;
- the posterior limb of the anterior commissure;
- the anlage of the corpus callosum.

The stage of 3 days postnatal

The further differentiation of various structures, among which the corpus callosum, the septal area, the thalamic region and the cerebral stem area, leads to a considerable reduction of the lumen of the lateral ventricle (c.f. plates 18 and 27).

A rather wide submaternal layer containing scattered mitotic figures is still present in this stage. Rostrally this layer is situated adjacent to the caudatus-putamen complex; more caudally the innermost bundles of the internal capsule pass through the submaternal layer. A similar close relation exists between the latter structure and the stria terminalis. This might suggest that the cell elements in question are involved in the myelination process of the fibres, which constitute these two bundles.

All of the nuclei constituting the strio-amygdaloid complex have increased in volume resulting in small changes in their topographical positions, however without loosing their mutual relationships (plate 28). Comparison of plate 18 with plate 28 reveals that both the dorsal, convex part and the ventral, flat part of the ventricular eminence correspond with parts of the caudatus-putamen complex. It can also be clearly observed that the stria terminalis follows the bended course of the sulcus terminalis. Undoubtedly there must be a correlation between the increased curvature of the stria terminalis and the further differentiation of the internal capsule. The latter is situated directly ventral to the stria, as can be seen in plate 27b. Finally, we will call attention to the expansion of the commissura

anterior. Its anterior and posterior limbs both first run laterally and then curve rostrally and caudally, respectively (plate 28).

Summarizing it can be stated that in comparison with the former stage the advanced differentiation of the various structures mainly results in a change of their topographic positions without affecting their mutual relationships.

The adult stage

In the adult stage the lateral ventricles are extremely narrow and in some places the walls of both sides even meet each other resulting in the so called coarctationes ventriculi (c.f. plates 19 and 29b). At the coarctations most of the ependymal layer has disappeared, sometimes a few ependymal cells persist arranged in rosettes or in irregularly shaped cell clusters.

As can be seen in plate 29a, a narrow subependymal layer, being the remainder of the submaternal layer, is present. This layer surrounds the dorsolateral angle of the lateral ventricle.

The ultimate spatial relationships of the various strio-amygdaloid components are shown in plate 30. Comparison of plates 19 and 30 reveals that the ventricular relief of the lateral ventricular surface can be correlated very well to the mantle layer derivatives, which in the adult stage are situated subjacent to the thin ependyma. This means that the rostral and medial parts of the curved ventricular eminence correspond to the caudatus-putamen complex, whereas the basolateral group of the amygdaloid complex protrudes somewhat into the lumen of the cornu inferius. The rest of the amygdaloid nuclei do not occupy a direct periventricular position.

The stria terminalis presents itself as a distinct bundle curving around the dorsal side of the cerebral stem region. Rostral to the foramen of Monro the stria is subdivided into three parts, which are named, according to their relation with the anterior commissure: the precommissural, the commissural and the postcommissural component.

Finally the anterior commissure itself should be mentioned. Its anterior limb can be followed up to the area retrobulbaris, whereas the posterior limb can be traced into the caudal parts of the cerebral hemisphere.

Comparison of plates 28 and 30 reveals that the differentiation process of the various mantle layer structures analysed continues even after the three days postnatal stage.

6.4 *Discussion and conclusions*

A fundamental property of the developing central nervous system is that its constituent cells originate within the matrix layer, which is situated along the ventricular surface. After being generated the cells either stay within the matrix layer and participate in the further proliferation or leave the matrix and loose their proliferative activity. The latter elements then migrate towards their ultimate position within the mantle layer and differentiate. However, at least in the telencephalon and probably also in other regions, a third possibility exists, namely that the cells leave the matrix layer maintaining their proliferative activity and constitute a submaternal layer at some distance from the ventricular surface. Thus the submaternal layer forms a second proliferative compartment. Subsequently, the cells generated in the submaternal layer may either retain their position and differentiate in situ or migrate to some other destination in the mantle layer to differentiate there. As a consequence in those regions in which a submaternal layer is present the developmental process of the mantle layer is very complex and rather difficult to interpret.

6.4.1 *The submaternal layer*

The submaternal layer, which is very prominent in the ventricular ridge area, like the matrix layer consists of mitotically active cell elements. However, contrary to the matrix cells, the submaternal cells are not radially arranged. Undoubtedly this phenomenon is related to the fact that the nuclei of the latter do not display an elevator movement (Sidman '70), whereas the nuclei of the matrix cells move to and from the ventricular surface. Secondly, the mitotic figures are randomly distributed over the entire submaternal layer contrary to the matrix layer where mitoses are found almost exclusively at the ventricular surface. Another striking difference between both layers is that the matrix layer exists already before the closure of the neural tube, whereas the submaternal layer originates in later developmental stages.

Finally a third remarkable difference between the matrix and the submaternal layer may be mentioned. The matrix layer eventually gets exhausted, although at different times in the various regions, and becomes transformed into the not mitotically active ependyma. On the other hand the subependymal layer, being the remnant of the submaternal layer, retains its proliferative potentiality even in the adult.

The great number of mitotic figures present within the submaternal layer indicates that this structure substantially participates in the production of cells. In early developmental stages the submaternal layer is probably involved in the formation of neuroblasts, whereas in later stages it mainly produces glial elements (Smart and Leblond,'61; Altman,'66). However, only a detailed investigation of the development of the submaternal layer might elucidate its role during ontogenesis.

6.4.2 *The mantle layer*

In early developmental stages the mantle layer, also that of the ventricular ridge area, originates directly external to the matrix layer. At that time the neuronal constituents of the mantle layer have undoubtedly been generated within the matrix layer, since a submaternal layer is not present as yet. However, in the 13.5 to 14 days stage a clear submaternal layer can be recognized in the medial and lateral ventricular ridge. Thus, theoretically, from that time onward each griseum which manifests itself in the mantle layer of the area in question may be constituted either by neurons derived from the matrix layer, or by neurons generated within the submaternal layer, or by neurons produced by both of these sources.

The mode of development of the various grisea can not be ascertained by determination of the time and site of their histological appearance, since such data are based upon the recognizability of differentiated neurons and therefore can only give information about the topographic relations of the developing grisea after their neuronal constituents have reached their ultimate destination. Secondly, it should be emphasized that the topographic position of the various grisea hardly gives any information concerning the site of origin of their constituent neurons.

6.4.3 *The histogenesis of the strio-amygdaloid complex.*

a. The globus pallidus.

In the stage of 13.5 days the first griseum within the region of the ventricular ridge area can be recognized: the globus pallidus formation. Its cells are closely related to the fibres which first appear in this region: the so called "Stammbündel" of His ('04). At the beginning of its differentiation, the globus pallidus is situated in the caudal part of the medial ventricular ridge at the level of the telodiencephalic boundary area. Thus it is located in that part of the ridge that extends into the wall of the third ventricle.

Additionally it has to be noted that it is closely related to the marginal zone at the level of the sulcus hemisphaericus. The close relationship with the fibres of the stembundle was already mentioned. These fibres pass from the diencephalic region to the ventricular ridge area. The cells of the globus pallidus in this stage are mostly spindle-shaped and arranged with their greatest diameter parallel to the fibres of the stembundle. This orientation suggests that the cells might have migrated along the fibres to a more rostral position.

Concerning the origin of the globus pallidus the following opinions have been expressed in the literature:

a) this structure originates entirely from the diencephalon (Spatz,'24, '25; J.E. Rose,'42; Starck,'55; Stroër,'56; Diepen,'62; Kahle,'51, '56, '69; Richter,'65, '66; Keyser,'72 among others).
b) this structure is a derivative of the telencephalon (Kodama,'26, '27; Ariëns Kappers,'23; Holmgren,'25; Grünthal,'41, '52; Källén,'51 a; Ranson and Clark,'53; Crosby, Humphrey and Lauer,'62).
c) the pallidum develops largely from the diencephalon, but its lateral part, which is bordered by the caudatus-putamen complex, originates probably from the medial ventricular ridge (Miura,'33 and Kuhlenbeck,'27, '30, '54).

The main reasons for assuming a diencephalic origin of the globus pallidus are as follows:

1) the pallidum differentiates early in development, synchronously with many diencephalic structures (Richter,'65; Keyser,'72, among others)
2) the constituent elements of the globus pallidus originate from the matrix of the so-called "Subthalamische Längszone" (i.e. the dorsal part of the hypothalamus region. This implies that the cells of the globus pallidus migrate from a caudal origin to a rostral position.

In our opinion the precocious differentiation of the globus pallidus does not preclude its telencephalic origin and it should also be pointed out that there is no direct proof of a (tangential) migration of cells from the subthalamic region up to the ventricular ridge in early stages of development (see also the autoradiographic data reported by Keyser,'72).

Nevertheless, we consider it quite possible that part of the globus pallidus is of diencephalic origin. Plate 21 shows that in the stages of 13.5 days the caudal part of the future globus pallidus is situated at the diencephalic level, whereas more rostrally the telencephalic region is reached, which would indicate a bipartite origin of the globus pallidus. In the next

chapter this problem will be further elaborated. It will be clear that in the discussion on the origin of the globus pallidus the definition of the telo-diencephalic border plays an important role.

After the stage of 13.5 days, the ventricular ridge area displays an extensive outgrowth, however, for some time no further mantle layer structures appear in this region. Only the globus pallidus can be delimited clearly up to the stage of 15 days, when the central nucleus of the amygdaloid complex appears. This late appearance of the mantle layer structures in the subpallium is in contrast with the differentiation of the diencephalon. Keyser ('72) has found that in the latter region as early as in the 14 days stage a number of grisea and fibre bundles can be distinguished.

b. The caudatus-putamen complex.

The caudatus-putamen complex can be considered as a rather late differentiating structure. This is in agreement with the observations of many other investigators (among them Hochstetter, '19; Ariëns Kappers, '23; Johnston, '23; Kuhlenbeck, '27; Hewitt, '58, Kahle, '69). In primates the complex is separated into the putamen (forming the lateral part of the nucleus lentiformis) and the nucleus caudatus, by fibres of the internal capsule. Some authors described one single anlage of the complex which is subdivided lateron into putamen and the nucleus caudatus (Spatz, '24; Löhe, '44; Kahle, '69). Others pointed out that the putamen would originate by a first proliferation wave of the matrix of the ventricular ridge area and that subsequently the nucleus caudatus is formed (Kodama, '26, '27). In lower mammals, as for instance the Chinese hamster, the putamen and nucleus caudatus can not be separated. The complex then must be regarded as a unitary structure of which the differentiation process continues after birth (cf plates 24, 26, 28, 30).

c. The amygdaloid complex.

As already mentioned before, the central amygdaloid nucleus is the first structure of the amygdala which can be recognized (15 days). This observation does not tally with the findings of Brown ('67) in bat embryos and Humphrey ('68, '72) in human embryos. These authors held that this nucleus appears after the nuclei corticalis and medialis amygdalae. Humphrey emphasized that the last finding would be in agreement with the phylogenetic appearance of the central nucleus as described by Crosby et al. ('66). These authors noted that the nucleus in question can not be recognized below reptiles. Contrary

to Humphrey's opinion Sidman and Angevine ('62, mouse) who studied the time of origin of the cells constituting the amygdaloid nuclei by autoradiography stated that the cells of the central and medial nuclei originate as the earliest in development, whereas later on the basal and cortical nuclei originate, followed by the lateral amygdaloid nucleus.

Concerning the appearance of the amygdaloid nuclei our observations indicate that all grisea of Johnston's ('23) corticomedial group can be recognized at the stage of 16 days except for the nucleus of the lateral olfactory tract, which we did not include in the corticomedial group. At this stage no subdivisions can be distinguished as yet in the anlage of the basolateral group. Thus, this group is somewhat retarded as compared with the corticomedial group. The suggestion of Johnston ('23) that the basolateral group would develop by an infolding of cellular elements from the external border of the brain by way of inward migration, has been rejected by some authors (Brown,'67; Humphrey,'68, '72). We agree with the opinions of the latter authors since we did not find any indication for such a migration.

As mentioned, the nucleus of the lateral olfactory tract, appears rather late in development as compared with the rest of the amygdaloid nuclei. This late appearance is also mentioned by other investigators (Johnston,'23; Brown,'67; Humphrey,'68, '72).

The other subnuclei of the amygdaloid rest group can only be recognized with certainty after birth (3 days postnatally). This phenomenon is obviously related to the rather vague histological characteristics of these grisea, the area amygdaloidea anterior and the massa intercalata are both ill defined regions even in the adult.

d. The fibre bundles.

The first fibre bundle to be recognized within the telencephalic area is the stembundle (Stammbündel of His). It is located in the telo-diencephalic transitional area and extends into the (medial) ventricular ridge. Later in development it can not be distinguished from the internal capsule as a separate tract. However, it can hardly be considered as a part of the internal capsule, because at the time of its appearance the thalamus dorsalis and the neocortex (two important sources of fibres of the internal capsule) have not differentiated as yet. The capsula interna proper appears in Chinese hamster embryos at the stage of 16 days. It passes from the diencephalon rostrally to the cerebral stem area and thence it bends laterally to the

subpallial region. In all stages studied the dorsal border of the capsule is roughly indicated by the concave stria terminalis which overcrosses it.

During further development a massive outgrowth of the internal capsule can be observed, especially after the stage of 18 days, i.e. during the period in which a strong differentiation of the neocortex occurs. The enormous expansion of this fibre mass causes an increase in volume of the cerebral stem region, which in its turn leads to the development of the inferior horn of the lateral ventricle (cf. chapter 4). It is obvious that the development of the internal capsule greatly influences the topographic relationships of the nuclear structures situated in the subpallium and the surrounding areas. It has already been indicated by Spatz ('27), Diepen ('62) and Richter ('65) that during development in this region important topographical shiftings occur among the nuclei.

Our reconstructions have shown that up to the 16 days stage the strio-amygdaloid structures together constitute a straight, rostrocaudally oriented column (plate 24). During further development the caudal part of this column, which contains the amygdaloid nuclei, arches ventrally (plates 26, 28, 30). It is, however, worth noting that this change in overall shape of the strio-amygdaloid complex does not influence the mutual relations of its constituent grisea. The course of the stria terminalis is clearly influenced by the expansion of the internal capsule. This stria, which represents an important afferent and efferent pathway of the amygdaloid complex, appears at the 16 days stage. Already in this stage it shows three components namely a precommissural, a commissural and a postcommissural one.

The time of appearance of this pathway suggests a close relation to the nuclei of the corticomedial group of the amygdala. This hypothesis is supported by experimental neuroanatomical data (see e.g. Cowan et al.,'65, Lammers,'72).

In intermediate developmental stages the stria terminalis is represented by a rather diffuse slightly curved tract which connects the amygdaloid complex with the preoptic and the rostral hypothalamic area. The structures mentioned are situated rather close to each other and both originate from the ventricular ridge area. During further development the stria terminalis becomes much longer and gets a more bended course.

The last fibre tract to be discussed is the anterior commissure. This tract can first be recognized at the stage of 16 days, although at that time only its anterior limb is present. It is interesting to note that the stria terminalis

sends a large bundle into the anterior commissure. At the stage of 18 days the posterior limb has appeared. This rather late development can be associated with the, also late, differentiation time of the grisea which are connected by this part of the commissure, being the neocortex and the putamen, as was demonstrated by the experimental neuroanatomical studies of Whitlock and Nauta ('65); Knook ('65) and van Alphen ('69). The author last mentioned described in addition a connection of the posterior limb with a part of the lateral amygdaloid nucleus, which is also a rather late developing structure.

7 THE TIME OF ORIGIN OF THE NEURONS CONSTITUTING THE STRIO-AMYGDALOID COMPLEX

Autoradiography

7.1 *Introduction*

In this chapter observations concerning the time of origin of the neurons constituting the various nuclei of the strio-amygdaloid complex, as obtained by the autoradiographic technique, will be presented. These data amplify the results of our analysis of the development of the grisea, presented in the preceding chapter. Secondly, a comparison of the time of origin of the neurons with the time of the first histological appearance of the various nuclei may yield information on the migration path of the pertinent neuroblasts.

7.2 *Literature*

The technical details concerning the preparation of autoradiograms, as well as the chemical aspects of the incorporation of radioactive thymidine into the cell nucleus, have been extensively discussed in the literature (Rogers, '73; Cleaver, '67; Schultze, '69; Sidman, '70). Here only the most critical steps in the development of the autoradiographic technique with regard to the study of the ontogenesis of the central nervous system will be mentioned.

In 1951 Reichard and Estborn demonstrated thymidine to be a specific precursor of deoxyribonucleic acid (DNA). Great progress was made in autoradiography when it proved to be possible to label thymidine with tritium, as was accomplished by Fitzgerald et al. ('51) and Robertson and Hughes ('59). Bennet et al. ('60) showed that DNA is very stable during cell life, because when a radioactive precursor was incorporated into the DNA of brain cells of rat embryos these cells retained the radioactivity for the whole life of the animal: thus, the cells had become permanently marked.

This particular property of brain cells has made it possible to determine the time of origin of neurons in the various parts of the neuraxis as for instance the cortex (Angevine and Sidman, '61; Berry and Rogers, '65), the hippocampus (Angevine, '65), the thalamus (Fernandez, '69; Angevine, '70) and the basal ganglia (Sidman and Angevine, '62).

These autoradiographic studies are based upon the following principle.

Dividing cells are labeled only if their premitotic phase coincides with the presence of radioactive thymidine, since in this period the cell duplicates its DNA and labeled thymidine will be incorporated. The label once taken up is fully retained throughout the cell life, that is to say if the cell does not undergo further division. In autoradiograms this results in the appearance of heavily labeled cells. However, if the cells do divide after the incorporation of radioactive thymidine, at each division the label is halved for each daughter cell. The cells which finally originate then display a weaker labeling than their precursor cells would do. Thus it may be concluded that heavily labeled cells originated at that time of development at which the radioactive material was administered, whereas weakly labeled cells apparently have passed one or more divisions. With regard to unlabeled cells it can be noted that they originated either before the administration of the labeled thymidine or after that time. In the last case the label has been diluted too much to demonstrate it in the autoradiograms either by many successive divisions, or by shrinkage of the thymidine precursor pool.

The cell birthday "is defined empirically on the base of autoradiographic data as the last day on which nuclear DNA is replicated in that cell line" (Sidman, '70). Generally it can be said that young neuroblasts which have left the matrix zone, do not divide anymore. If tritiated thymidine is present at the time at which the neuron precursor cell prepared its division into two neuroblasts, the thymidine will be incorporated into the DNA which will be divided over both daughter cells. Thus these cells are marked during their further life, because their stable chromosomal DNA is labeled and can be demonstrated autoradiographically. In this way the neuron birthday can be determined.

The determination of the cell-birthday can only be carried out by a "flashlabeling" procedure. This manner of administration of the radioactive thymidine implies that it is only available for incorporation during a short time (about 1 to 2 hours), in order to achieve that the heavily labeled cell population will be as small as possible.

As was indicated in chapter 2, the observations to be described are obtained from a great number of series of adult animals to which tritiated thymidine was administered by way of flash labeling at different developmental stages. In this chapter an attempt will be made to get an answer to the following questions:

- is the time of the first histological recognition of the grisea of the strio-amygdaloid complex (as described in the preceding chapter) in accordance with the time of origin of their constituent neurons (as obtained by the autoradiographic technique)?
- is it possible to demonstrate the existence of certain developmental gradients within the grisea (for instance inside-out or outside-in)?
- can a comparison of the times of origin of the neurons constituting the nuclei with the matrix activity of the various regions provide some information concerning the sites of origin of those neurons?

7.3 *Observations*

The adult Chinese hamsters used are animals to which tritium thymidine was administered during the embryonic period ranging from the developmental stages of 12 to 18 days post conception, as described in chapter 2.

With regard to the illustrations it has to be noted that only the heavily labeled cells were plotted.

The globus pallidus

Histologically the globus pallidus appears at the stage of 13.5 days, forming part of the mantle layer of the ventricular ridge area.

The autoradiographic results demonstrate that the neurons of the medial part of the globus pallidus get labeled on embryonic day 12 and 13, as can be seen in figure 21 and 22. The lateral part of the globus pallidus shows many heavily labeled neurons after injection on day 14 (fig. 23). These data suggest that the neurons constituting the globus pallidus are deposited in a medio-lateral or inside-outside fashion. This phenomenon will be discussed later on.

The caudatus-putamen complex

Histogenetically the caudatus-putamen complex appears at a rather late stage of development. In Chinese hamster embryos it is recognizable on embryonic day 15. However, one day later the complex can first be delimited.

In autoradiography the first heavily labeled cells in this complex can be observed after administration of thymidine on embryonic day 14 (figs. 23 and 24). The labeling reaches a peak on day 15 and 16 (figs. 25, 26 and 27), whereas on day 17 some more neurons become labeled (fig. 28). In our material

no clear pattern of labeling suggesting a certain developmental gradient, could be observed.

The corticomedial cell group of the amygdaloid complex

Histologically, the grisea of this group can first be delimited at the stage of embryonic day 16. However, it must be mentioned that the central nucleus which appears first, is indicated somewhat earlier, namely on day 15.

The autoradiographic material shows that the neurons of each of the grisea of this group display a strong labeling in those animals to which thymidine was administered on embryonic day 12 and 13 (figs. 21 and 22). On day 14 less cells become heavily labeled (fig. 24). Thus it can be concluded that most neurons of the corticomedial group arise before day 14. Secondly, the uniformity of all grisea of this cell group as far as the time of origin of their neurons is concerned should be noted.

The basolateral group of the amygdaloid complex

In the preceding chapter it was described that the basolateral group first can be recognized on embryonic day 16. However, at that time a subdivision into the nucleus basalis and lateralis is hardly possible. The latter nucleus can only be delimited with certainty from day 18 on.

Heavily labeled cells within the basolateral group can be observed in animals which were injected on embryonic day 14 (figs. 23 and 24). This holds both for the nucleus basalis and the nucleus lateralis.

Thus it appears that the first neurons of both parts of this group arise simultaneously. During further development more neurons originate up to embryonic day 16 (figs. 25, 26 and 27), and even after administering thymidine on day 17 a few cells in the complex still show a heavy labeling (fig. 28). The position of these last cells is in the neighbourhood of the lateral ventricle. The rostral part of the group gets labeled somewhat earlier than the caudal part. These phenomena suggest a certain developmental gradient of the basolateral group, namely from a rostral to a caudal level. No clear developmental gradient could be observed with regard to the lateral and medial parts of the group.

The restgroup of the amygdaloid complex

As was described before, the grisea of this group can histologically be

recognized at a rather late stage of development: embryonic day 18 and after birth. Partly this is due to the fact that two grisea, namely the area amygdaloidea anterior and the massa intercalata can hardly be delimited even in the adult stage. For this reason it is important to know in which developmental period the neurons of these grisea arise.

The area amygdaloidea anterior already shows a rather heavy labeling when injection occurs on day 12 (fig. 21). Similar results are obtained at 13 and 14 days (figs. 22 and 23). The cells of the intercalate mass arise somewhat later. Although not indicated in the figures, its labeling reaches a peak on day 14 and 15. Concerning the nucleus of the lateral olfactory tract it can be noted that this nucleus displays a few heavily labeled cells on day 13. A more extensive labeling is observed on day 14 and 15 (figs. 23 and 25). Thus the time of origin of the neurons of the area amygdaloidea anterior is somewhat earlier than those of the other two subdivisions of the rest group.

7.4 *Discussion*

The autoradiographic data about the time of origin of the neurons constituting the nuclei of the strio-amygdaloid complex in the Chinese hamster are summarized in table 4, together with the time of first histological appearance of these nuclei, as described in the preceding chapter. For comparison the time of origin of the neurons of the various nuclei in the mouse, as found by Sidman and Angevine ('62), are also included.

Taking into account that the gestation period in the Chinese hamster and in the mouse lasts 21 and 19 days respectively, the results roughly correspond with each other. The only notable difference is that according to Sidman and Angevine the neurons of the nucleus corticalis originate later than those of the other two nuclei of the corticomedial group of the amygdaloid complex whereas our results show that the neurons of all three nuclei arise at the same developmental period. The authors mentioned did not include the restgroup of the amygdaloid complex in their investigation. However, Creps ('74) described that the neurons of the nucleus tractus olfactorius lateralis in the mouse originate on embryonic day 12-13, which corresponds with embryonic day 14-15 in the Chinese hamster.

As regards the globus pallidus neurons our autoradiographic data show that they can be subdivided into two categories: those having an early time

	Chinese hamster		Mouse (Sidman and Angevine, '62)
	Time of origin of the neurons	Time of first histological appearance	Time of origin of the neurons
Globus pallidus	12-14	13.5	(10) 11 (12-13)
Caudatus-putamen complex	(14) 15-16 (17)	15-16	12-15
Corticomedial group			
nc. centralis	12-13 (14)	15	10-11
nc. medialis	12-13 (14)	16	10-11
nc. corticalis	12-13 (14)	16	(11) 12-13
Basolateral group			
nc. basalis	14-16 (17)	16	(11) 12-13
nc. lateralis	14-16 (17)	16	(10) 12
Rest group			
nc. tractus olfactorius lateralis	(13) 14-15	18	-
area amygdaloidea anterior	12-14	(18) 3 pn	-
massa intercalata	14-15	(18) 3 pn	-

Table 4: Time of origin of the grisea constituting the strio-amygdaloid complex

of origin and others arising somewhat later. The latter group is principally situated in the outer part of the pallidum, which is bordered by the caudatus-putamen complex. In the preceding chapter it was pointed out that the globus pallidus might have both a diencephalic and a telencephalic origin, as was already suggested by Miura in 1933. The autoradiographic results support this view. If this hypothesis is correct the main part of the globus pallidus has a diencephalic origin, whereas its lateral part arises from the telencephalic anlage. Possibly, the neurons of the latter part originate within the submaternal layer of the ventricular ridge area.

Before comparing the time of origin of the neurons constituting the various strio-amygdaloid nuclei with the time of the histological appearance

of these nuclei some general comments should be made. Based upon findings in Golgi material Morest ('69a, '70) has stated that the final morphological differentiation of neurons starts when the cell elements are in their post-migratory phase. If this conclusion is correct the period between the production of the neuroblasts that are going to constitute a given cell mass and the actual appearance of that cell mass may be characterized as the migratory phase of these neuroblasts. The duration of the migratory phase is mainly determined by the length of the migration path and the velocity of migration. During development the factors just mentioned presumably increase and decrease, respectively. Thus, generally, the neuroblasts which arise later in development will show a longer migratory phase than those which originate earlier. On that account some assumptions can be deduced from the data as summarized in table 4.

1. Both the globus pallidus and the caudatus-putamen complex show that their constituent neurons start to differentiate almost immediately after their origin indicating that their migratory phase must be very short. Thus, it may be assumed that a close topographical relationship exists between the postmigratory location of these grisea and their respective matrix areas.
2. The neurons of the corticomedial group of the amygdaloid complex show a long migratory time although they originate in an early developmental period. Thus, their neurons probably cover a long distance from their site of origin within the matrix to their ultimate position.
3. As regards the basolateral group of the amygdaloid complex it is most likely to assume that the matrix area in which its neurons originate is located close to their final position.
4. The restgroup of the amygdaloid complex shows a long period between the time of origin of the neurons and the time of first histological appearance of the grisea, suggesting a long migratory route. This holds in particular for the nucleus tractus olfactorius lateralis. The area amygdaloidea anterior and the massa intercalata are of a rather diffuse appearance and this may delay the time of first recognition of the grisea.

However, it should be realized that in the present investigation the time of first appearance of the grisea is based on the study of hematoxylin and eosin and Nissl preparations. Possibly, material of this type is not suited for an exact determination of the start of the differentiation of the neurons. Moreover, the migratory phase of the neuroblasts might be influenced by other

factors than the length of the migratory path and the velocity of migration. In spite of these uncertainties the data presented above can be very useful in determining the site of the matrix areas in which the neurons constituting the various grisea do originate, as will be elaborated in the next chapter.

Autoradiography.

Figures 21 - 28

fig. 21 Transverse section showing the position of heavily labeled neurons in the strio-amygdaloid complex in the adult after injection of ^{3}H-thymidine on embryonic day 12.

fig. 22 Transverse section showing the position of heavily labeled neurons in the strio-amygdaloid complex in the adult after injection of ^{3}H-thymidine on embryonic day 13.


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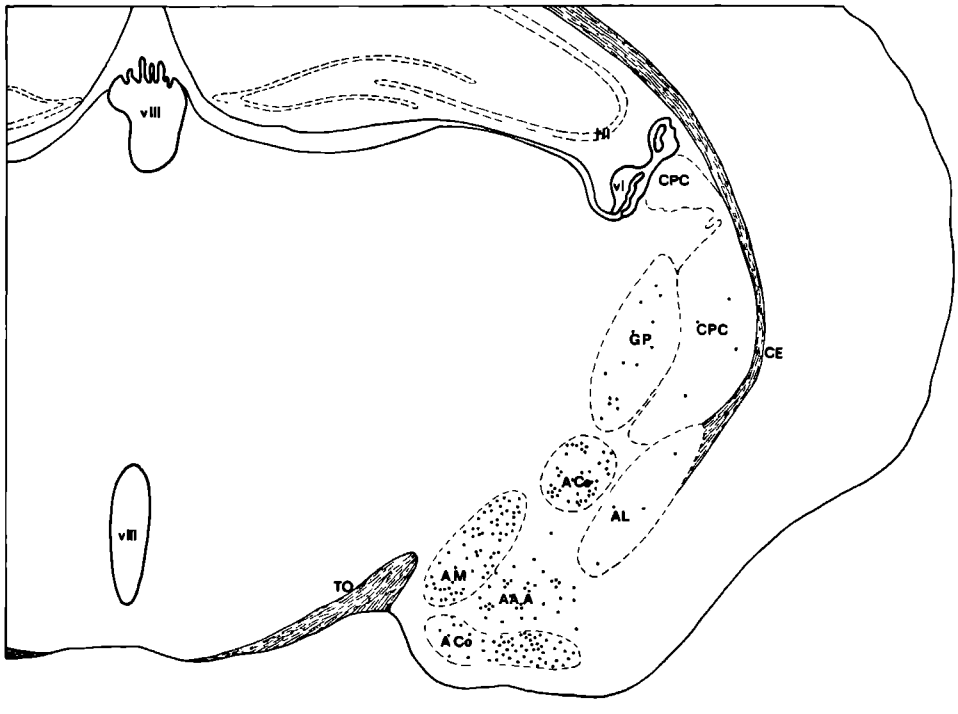
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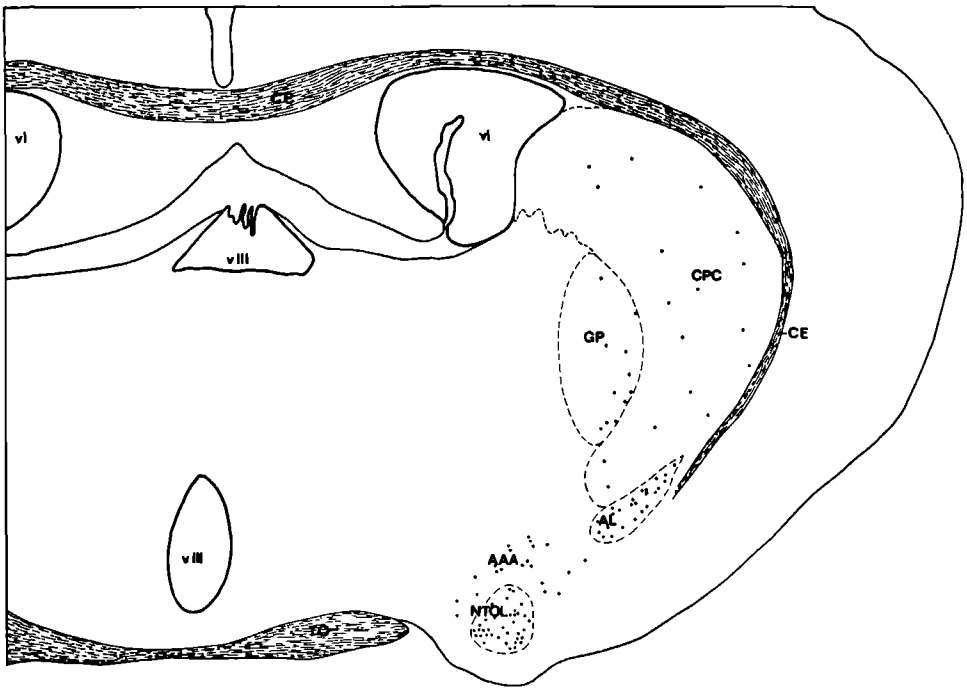
fig. 23 Transverse section showing the position of heavily labeled neurons in the strio-amygdaloid complex in the adult after injection of ^{3}H-thymidine on embryonic day 14 (rostral level).

fig. 24 Transverse section showing the position of heavily labeled neurons in the strio-amygdaloid complex in the adult after injection of ^{3}H-thymidine on embryonic day 14 (caudal level).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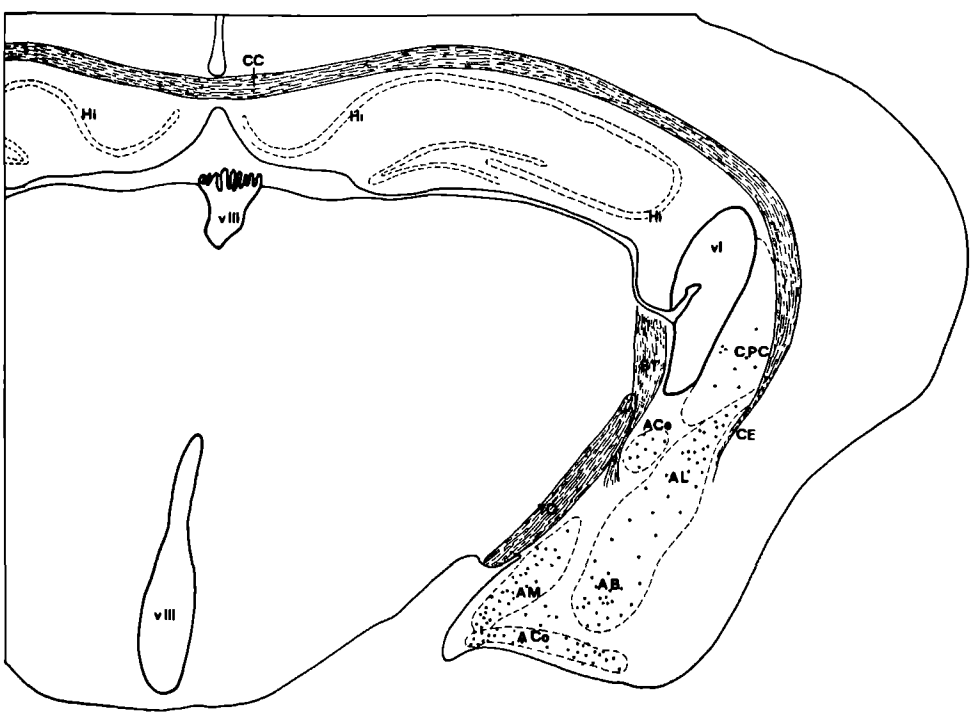
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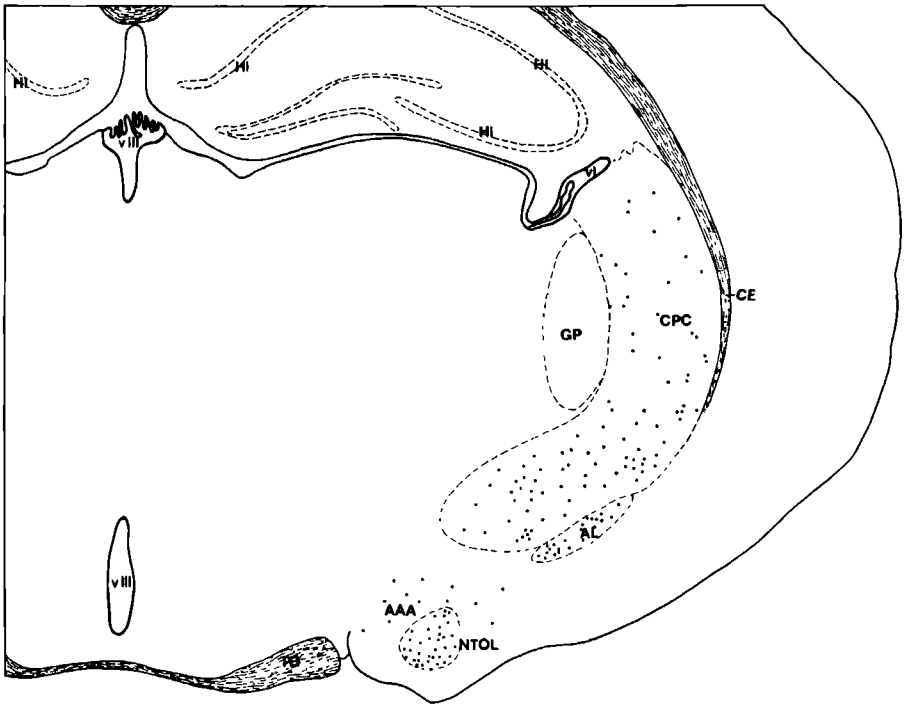
fig. 25 Transverse section showing the position of heavily labeled neurons in the strio-amygdaloid complex in the adult after injection of ^{3}H-thymidine on embryonic day 15 (rostral level).

fig. 26 Transverse section showing the position of heavily labeled neurons in the strio-amygdaloid complex in the adult after injection of ^{3}H-thymidine on embryonic day 15 (caudal level).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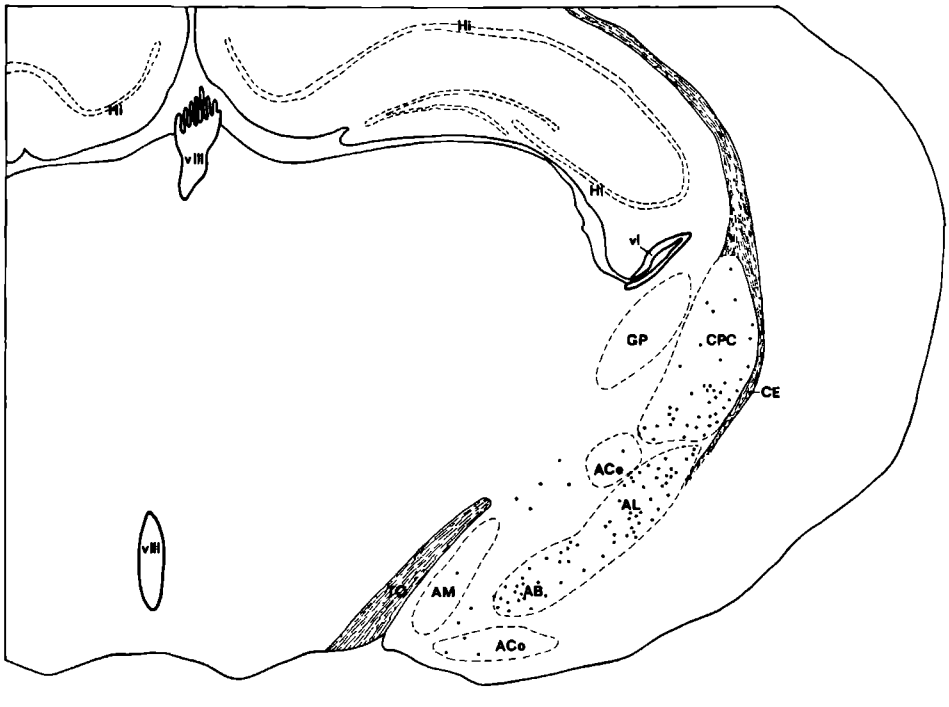
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fig. 27 Transverse section showing the position of heavily labeled neurons in the strio-amygdaloid complex in the adult after injection of ^{3}H-thymidine on embryonic day 16.

fig. 28 Transverse section showing the position of heavily labeled neurons in the strio-amygdaloid complex in the adult after injection of ^{3}H-thymidine on embryonic day 17.


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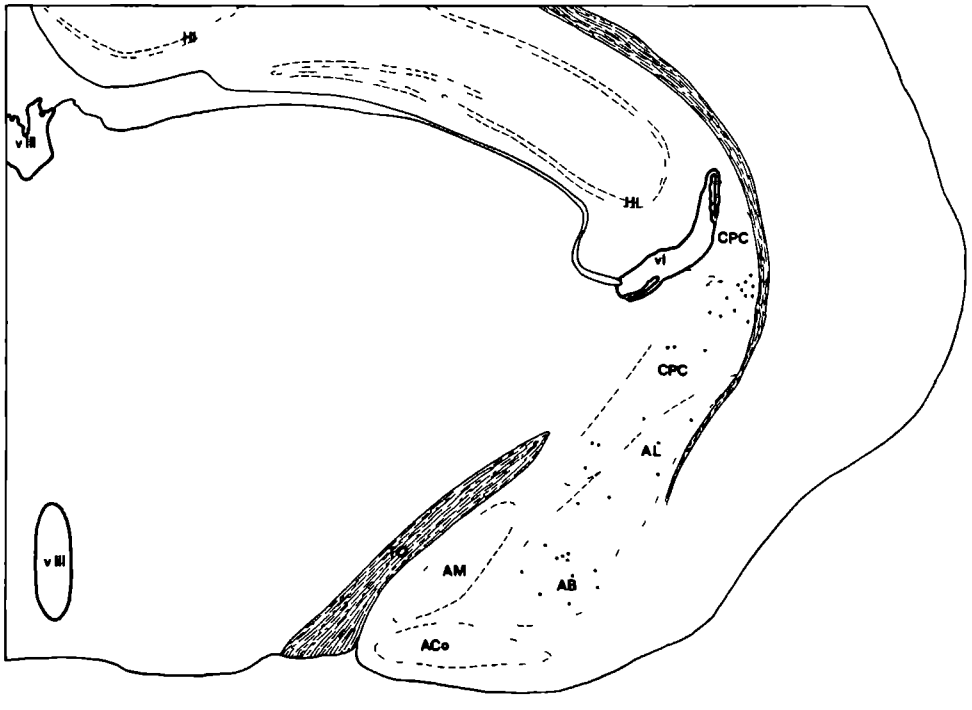
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8 GENERAL DISCUSSION

8.1 *Introduction*

In the preceding chapters the three main aspects of the ontogenesis of the strio-amygdaloid complex, being the morphogenesis, the matrix development and the histogenesis of the mantle layer structures, have been dealt with. In the ensuing chapter an attempt will be made to give a synthetic review of the results obtained.

In the first part the morphogenesis of the ventricular ridge area will be discussed in the light of the processes occurring in the wall of the neural tube. Special attention will be paid to the relations between morphological transformations on the one hand and the development of the matrix and the submaternal layer on the other hand.

In the second part the histogenesis of the grisea constituting the strio-amygdaloid complex will be considered in comparison with the development of the matrix layer of the various areas. This approach might provide some evidence concerning the site of origin in the matrix layer of the neuronal constituents of these grisea.

8.2 *The morphogenesis of the ventricular ridges*

At the stage of 12 days and 7 hours the rostral part of the neural tube consists of a matrix layer and an outer marginal layer, displaying phase 0 of matrix development. The inner and outer surface of the rostral part of the neural tube are almost parallel to each other. At the ventricular side the telodiencephalic boundary is clearly marked by the torus hemisphaericus, which is the counterpart of the sulcus hemisphaericus on the outside. Basally and rostrally this torus fades towards the torus transversus (plate 1).

At the 12.5 days stage two centres of advanced matrix development can be observed (fig. 11), the one is situated in the hypothalamic region and corresponds with the hypothalamic cell cord of Gilbert ('35), the other is found in the telodiencephalic border zone, ventrally to the foramen of Monro. In both areas the advanced matrix development is accompanied by a local thickening of the wall of the neural tube. Due to the rapid progress of the matrix activity basally to the foramen of Monro the basal part of the torus hemisphaericus is replaced by the first anlage of the medial ventricular

ridge (plate 2). As a consequence, the telodiencephalic boundary can not be indicated any longer, since the medial ventricular ridge is mainly situated at the telencephalic level but also extends into the diencephalic wall (fig. 6). All other areas analysed still show a thin walled condition. At the stage of 13 days the proliferation in the matrix of the lateral ventricular ridge area has led to the formation of a slight thickening of the wall (cf. figs. 12 and 7 and plates 3 and 4). This first anlage of the lateral ventricular ridge is completely separated from the medial ventricular ridge by the sulcus subpallii intermedius. Meanwhile the medial ventricular ridge is protruding far into the ventricular space. This phenomenon is not only related to the intrinsic and extrinsic activity of the matrix layer but also to the cell production within the submaternal layer, which in this stage is already present at the level of the medial ventricular ridge. In the 13.5 days stage the matrix of the medial ventricular ridge shows a high intrinsic and extrinsic activity (fig. 13). Besides a wide submaternal layer contributes to the expansion of the medial ventricular ridge, which leads to a reduction of the foramen of Monro (plate 6).

The matrix development of the lateral ventricular ridge area shows some progress as compared with the preceding stage, which results in the morphological configuration as represented in plates 7 and 8. Rostrally both ridges are separated by the sulcus subpallii intermedius, but caudally this sulcus has disappeared. In our opinion the unification of the caudal parts of both ridges occurs by fusion of the cellular material underneath the matrix layer. The matrix of the pallial region is retarded as compared with that of the lateral ventricular ridge. A distinct ventricular groove, the sulcus subpallii dorsalis, marks the boundary between the pallium and the lateral ventricular ridge.

At 14 days the phenomena described above are even more accentuated. The matrix of the lateral ventricular ridge area, although retarded as compared with that of the medial ventricular ridge, shows a high intrinsic and extrinsic activity. Moreover, from this stage on a clear submaternal layer is also present in this region. The cell production in both layers thus contributes to the protrusion of the lateral ventricular ridge into the lateral ventricle. The fusion of both ridges which started caudally at 13.5 days has proceeded rostrally: only the most rostral parts of the ridges are still separated by the sulcus subpallii intermedius. The matrix condition of the caudal, unified

part is similar to that of the lateral ventricular ridge. However, the continuity between the submaternal layers of both ridges indicates that the increase of cellular elements in this layer may be responsible for the unification process. The sulcus subpallii dorsalis has become a rather deep groove as a result of the progressive expansion of the lateral ventricular ridge into the ventricular cavity in contradistinction to the pallial region. At the stage of 16 days the matrix of the lateral ventricular ridge area has reached the same phase, i.e. the phase of full migration, as that of the medial ventricular ridge (figs. 14, 17). As a consequence histologically both ridges show a uniform condition. Moreover, in both ridges a wide submaternal layer is present as can be seen in plate 23. Morphologically, both ridges are almost one entity except for their most rostral extensions which are still separated by the remaining part of the sulcus subpallii intermedius (plates 14, 15, 16). Although the intrinsic activity of the matrix layer in the pallial region is somewhat higher than in earlier stages, this region is still thin-walled as compared to the ventricular ridge area. Thus the borderline between both areas remains marked by a rather deep sulcus subpallii dorsalis. At the 18 days stage the matrix of the ventricular ridge area displays a phase of exhaustion (figs. 18, 19). This implies that its intrinsic activity is rather low and that in this stage most of the cell production occurs within the submaternal layer. Neither the submaternal layer nor the matrix layer of the ventricular eminence show any sign of their dual origin (plate 25). Morphologically one single ventricular eminence is present; the sulcus subpallii intermedius has completely disappeared (plate 17). The matrix of the pallial region now shows the phase of full migration, causing a thickening of the wall in this area. This leads to a shallowing of the sulcus subpallii dorsalis. However, this sulcus remains present, as can be seen in plates 17 and 25.

During further development the morphological pattern, as described in the 18 days stage, only shows minor changes. Summarizing the processes described above it can be stated that the progressive matrix development of the medial ventricular ridge area results in a local thickening of the wall, which is bordered by the sulcus subpallii intermedius. Subsequently, the matrix activity in the lateral ventricular ridge area causes a second thickening of the wall, which is accompanied by a deepening of the sulcus subpallii intermedius and the development of the sulcus subpallii dorsalis. In the medial ventricular ridge a submaternal layer originates, which after some time also contri-

butes to the expansion of this structure. Similar events occur somewhat later in the lateral ventricular ridge. After the submaternal layers of both ridges have become continuous, they produce such an amount of cellular material that the sulcus subpallii intermedius fades. This process starts caudally and proceeds rostrally. The matrix layer then also shows a uniform condition, which leads to the formation of one single ventricular eminence. The sulcus subpallii dorsalis, however, remains, since the matrix layers of the pallial region and the lateral ventricular ridge area retain a different condition and, moreover, in the pallial region no submaternal layer develops.

8.3 *The histogenesis of the grisea constituting the strio-amygdaloid complex*

In the preceding chapter it has been pointed out that comparison of the time of first histological appearance of the various nuclei with the time of origin of their constituent neurons yields some information concerning the migration path of the respective neuroblasts. In this way the distance between the postmigratory position of each griseum and the matrix area in which its neurons probably originate could be indicated. In the ensuing description an attempt will be made to determine more specifically the matrix area "belonging" to each griseum. This is accomplished by analysing the developmental condition of the various matrix areas at the time of origin of the neurons constituting the various grisea.

8.3.1 *The striatum*

The globus pallidus

The globus pallidus is the first griseum which histologically can be recognized, namely at the 13.5 days stage. It is located at the telodiencephalic border zone within the mantle layer of the medial ventricular ridge area and shows a close topographical relationship with the submaternal layer (cf. plates 20 and 21). The autoradiographic data demonstrate that the neurons constituting the globus pallidus are generated during the period between embryonic day 12 and 14. However, it should be noted that the neurons, arising on day 14, are located within the lateral part of the globus pallidus.

As was pointed out in the preceding chapter the postmigratory position of the globus pallidus appears to be close to its original matrix area. Analysis of the matrix development during day 12 through 14 reveals that at that

time two areas show a clear extrinsic matrix activity. The first is located at the diencephalic level, namely the region of the hypothalamic cell cord of Gilbert ('35). It may be assumed that the neurons of the main part of the globus pallidus, which arise earliest, are generated within this area. These neurons, therefore, have a diencephalic origin and probably migrate tangentially in a rostral direction. This hypothesis is also supported by the fact that the cells are orientated with their long axis parallel to the fibres of the stem bundle.

The second matrix area which may be involved in the production of globus pallidus neurons is located in the medial ventricular ridge area. However, the extrinsic activity of this matrix area probably mainly results in an accumulation of cells in the submaternal layer. After one or more mitotic divisions, these submaternal cells may migrate towards their final destination. If this hypothesis is correct, only those neurons of the globus pallidus, which arise later in development originate in this way. Hence it seems probable that the neurons of the lateral part of the globus pallidus, which arise on embryonic day 14, are generated within the matrix of the medial ventricular ridge area. Whether these neurons have a diencephalic or a telencephalic origin can not be determined, since a clear telodiencephalic boundary is not present.

It has already been mentioned that most authors propose either a diencephalic origin of the globus pallidus (Spatz, '24; M. Rose, '35; Richter, '65, '66; Kahle, '69 and Keyser, '72) or a telencephalic one (Ariëns Kappers, '23; Kodama, '26, '27; Grünthal, '52). In our opinion the globus pallidus has a dual origin as was also suggested by Miura ('33) and Kuhlenbeck ('54).

The caudatus-putamen complex

Histologically the caudatus-putamen complex first appears on embryonic days 15-16. It is located within the mantle layer of the medial as well as the lateral ventricular ridge area (cf. plates 22, 23 and 24). Topographically the complex is closely related to the submaternal layer and especially during its first differentiation period it is hardly possible to indicate a border between these two structures.

Most of the neurons constituting the caudatus-putamen complex are generated on embryonic days 15 and 16, as was autoradiographically established (figs. 25, 26 and 27). Since the differentiation of the complex also starts on embryonic days 15-16, it can be concluded that the migratory phase of its

neurons is very short. Apparently, the post-migratory position of the caudatus-putamen complex is close to the matrix area in which its neurons are generated. Topographically, the matrix of both the medial and lateral ventricular ridge area has to be considered as the site of origin of the neurons constituting the complex under discussion. At the stage of 16 days both matrix areas show a high extrinsic activity (figs. 16, 17). However, it has to be noted that probably most of the cell elements arising at that time enter the submaternal layer, which forms a single proliferative compartment throughout both ventricular ridges (plate 23). Because of the close topographical relationship between the differentiating caudatus-putamen complex and the submaternal layer, it is most likely to suppose, that the neuroblasts, which ultimately form the caudatus-putamen complex, undergo their final mitotic division within the submaternal layer, but primarily are generated within the matrix layer of both ventricular ridges. This conclusion is at variance with that of some other authors, among them Johnston ('23), Kodama ('27) and Kahle ('69), who suggested that the neurons constituting the caudatus-putamen complex originate only from the matrix layer of the lateral ventricular ridge.

8.3.2 *The amygdaloid complex*

The corticomedial group

From the grisea constituting the corticomedial group of the amygdaloid complex the nucleus centralis differentiates first, namely on embryonic day 15. The nucleus corticalis and the nucleus medialis both appear on embryonic day 16 (plate 24). At that time the corticomedial group is situated within the mantle layer of the caudal, undivided part of the ventricular ridge area. The autoradiographic data demonstrate that the neurons of all three corticomedial nuclei are generated in a much earlier developmental period namely on embryonic days 12-13 (figs. 21, 22). This considerable difference between the time of generation and histological appearance suggests that the neuroblasts involved have a rather long migratory phase. Morphologically, the anlage of the medial ventricular ridge is present on embryonic day 12.5 (plate 2 and fig. 6), whereas at 13 days the lateral ventricular ridge begins to appear (plates 2, 3 and fig. 7).

As regards the matrix development the only part of the matrix layer within the ventricular ridge area which shows a rather obvious extrinsic activity on embryonic day 12.5 and 13 is located in the medial ventricular

ridge (figs. 11, 12). From these data it can be concluded that all neurons constituting the grisea of the corticomedial group of the amygdaloid complex are generated within the matrix layer of the medial ventricular ridge. The rather long migratory phase of the neuroblasts involved could be explained by assuming that after their origin these elements remain for some time within the submaternal layer. However, at the stages of 12.5 and 13 days a submaternal layer is hardly present. Moreover, the very early labeling excludes any further mitotic division. This renders a long stay within the submaternal layer very unlikely. In our opinion the long duration of the migratory phase has to be attributed mainly to the length of the migratory path, since the corticomedial nuclei are ultimately located at a considerable distance from the ventricular surface (cf. plates 23, 25).

The basolateral group

Both the nucleus basalis and the nucleus lateralis amygdalae start to differentiate on embryonic day 16, i.e. at almost the same time as the grisea of the corticomedial group. At that time the anlage of the basolateral group is also situated within the mantle layer of the caudal undivided part of the ventricular ridge area. However, contrary to the corticomedial group the basolateral group shows a close topographical relationship with the submaternal layer (plate 23). A second remarkable difference between both groups of nuclei can be deduced from the autoradiographic data. The neurons of the corticomedial group are generated on embryonic day 12-13, whereas those of the basolateral group mainly arise on embryonic days 14-16 (figs. 23-27). It can be concluded that the neuroblasts forming the nuclei of the basolateral group contrary to those of the corticomedial group, start to differentiate almost immediately after their origin. Thus, the ultimate position of the basolateral group will be in the vicinity of the site of the origin of the respective neuroblasts. The close topographical relation of the basolateral group with the submaternal layer renders it likely that the last mitotic division of the neuroblasts forming this group occurs within the submaternal layer.

As regards the matrix development, the caudal, undivided part of the ventricular ridge area mainly resembles the lateral ventricular ridge. This suggests that primarily the cells which ultimately result in the neurons of the basolateral group originate from the matrix of the lateral ventricular

ridge. However, the material available for the present study did not allow definitive conclusions at this point.

The restgroup

Histologically, the nucleus tractus olfactorius lateralis, the area amygdaloidea anterior and the massa intercalata, forming the grisea of the restgroup of the amygdaloid complex, can first be recognized at a rather late developmental period, namely between embryonic day 18 and 3 days postnatally. The nucleus tractus olfactorius lateralis is situated at the rostrobasal part of the amygdaloid complex (plates 26 and 28). The area amygdaloidea anterior and the massa intercalata are hardly delimitable. Topographically, they are closely related to the nucleus tractus olfactorius lateralis.

As was established autoradiographically, the neurons of the nucleus tractus olfactorius lateralis arise on embryonic days 14-15 (figs. 23 and 25), like those of the massa intercalata. Morphogenetically, at that time the rostral parts of the two ventricular ridges are separated by the sulcus subpallii intermedius (cf. plates 11 and 16). The topographical position of both grisea indicates that they probably have to be related to the medial ventricular ridge. On embryonic days 14-15 the matrix of the medial ventricular ridge shows a high extrinsic activity (figs. 14 and 17), however, it has to be emphasized that a wide submaternal layer is present. On that account it might be suggested that the neurons of both the nucleus tractus olfactorius lateralis and the massa intercalata arise within the submaternal layer, although a direct contribution of the medial ventricular ridge can not be excluded.

The area amygdaloidea anterior differs from the other two restgroup components, as far as the time of origin of its neurons is concerned. Its neurons arise earlier, namely on embryonic days 12-14 (figs. 21, 22, 23). An analysis of the matrix development during that period shows that the matrix of the medial ventricular ridge exhibits a rather high extrinsic activity (figs. 11-14). Since the submaternal layer only develops after 13 days, it may be concluded that the neurons of the area amygdaloidea anterior are generated within the matrix of the medial ventricular ridge. However, the submaternal layer possibly contributes to those neurons of the area amygdaloidea anterior which arise after embryonic day 13.

In the present study the morphogenesis and the histogenesis of the strio-amygdaloid complex in the Chinese hamster have been analysed.

In this animal gestation lasts twenty-one days. The developmental period analysed ranges from the moment of initial evagination of the cerebral hemispheres (i.e. approximately embryonic day 12) to the adult stage. The material, consisting of closely graded staged embryos and animals, aged three and hundred days postnatally, was serially sectioned and stained according to the conventional neuro-anatomical techniques. The interpretation and the visualization of the morphogenetic and histogenetic results were facilitated by the use of graphical as well as three-dimensional reconstructions.

The analysis of the functional state of the matrix layer was based upon a subdivision of the developmental process of the neuroepithelium into a number of subsequent phases. In order to establish the time of origin of the neurons, constituting the various grisea of the strio-amygdaloid complex, the autoradiographic technique was employed. The results of this investigation can be summarized as follows.

The morphogenesis of the basal and lateral telencephalic wall is characterized by the development of two ventricular ridges. The medial ventricular ridge originates first at the level of the torus hemisphaericus, thereby obscuring the basal part of the telo-diencephalic boundary. Subsequently, the lateral ventricular ridge arises. Originally, both ridges are completely separated by the sulcus subpallii intermedius. During further development, however, this limiting groove fades away, starting caudally and gradually proceeding in the rostral direction. Eventually, this process results in the formation of one single ventricular eminence. In none developmental period a third ventricular ridge could be discerned.

The progressive protrusion of the medial ventricular ridge into the ventricular lumen, combined with the outgrowth of the eminentia thalami as well as the torus transversus, cause a reduction of the foramen of Monro into a small cleftlike opening. As a consequence, the medial part of the medial ventricular ridge, which ultimately gives rise to the preoptic region, finally holds an isolated position. The expansion of the cerebral stem area, in which the ventricular eminence is involved, ultimately results in a curvature of the ventricular eminence and in the formation of the cornu inferius of the lateral ventricle. The radial outgrowth of the pallial area, which is initiated after

the unification of both ventricular ridges, considerably reduces the lumen of the lateral ventricle. In the adult stage the latter is characterized by the presence of coarctation areas and the absence of a ventricular lumen within the olfactory bulb. In broad outline the results of the analysis of the functional state of the matrix layer during development show the presence of both a caudorostral and a basodorsal gradient as described by other authors (cf. His, '04; Spatz, '27 and Kahle, '51, '56, '58, '69). More in detail, however, the developmental pattern of the prosencephalic matrix is more complicated, as was also shown by Keyser ('72). The increase of the activity of the prosencephalic matrix is initiated at two centres, roughly corresponding with the hypothalamic cell cord and the medial ventricular ridge area, respectively. Subsequently, the developmental progress spreads from these centres to the surrounding areas. Within the lateral telencephalic wall the developmental pattern of the matrix layer shows a clear organization into longitudinal zones. In the preoptic region the developmental process of the matrix is initiated somewhat later but proceeds faster than in the medial ventricular ridge area. On that account, in the preoptic region the matrix is exhausted prior to that of the medial ventricular ridge. The matrix of the latter area attains its height earlier than that of the lateral ventricular ridge. However, at the moment of unification of both ridges the development of the matrix of the lateral ridge is in the same phase as that of the medial ridge. From that time onward the matrix of the entire ventricular eminence develops almost uniform up to its exhaustion. The development of the matrix layer in the pallial region lags far behind as compared with that of the ventricular ridge area. Summarizing, it may be stated that generally the developmental pattern of the matrix layer anticipates the morphological pattern.

The heterochronous development of the matrix layer is reflected by the sequential formation of the mantle layer structures. Of the grisea constituting the strio-amygdaloid complex the globus pallidus is the first to arise. This nucleus starts to differentiate almost immediately after the origin of its constituent neurons. The neurons of the medial part of the globus pallidus probably arise at the diencephalic level, migrating rostrally in a tangential way. The neurons of its lateral part seem to be generated within the submaternal layer belonging to the medial ventricular ridge. The topographical position of the caudatus-putamen complex as well as the time of origin of its neurons strongly suggest that the latter are derivatives of the

submaternal compartment extending over both ventricular ridges.

The histogenesis of the three main groups of amygdaloid nuclei is rather different. The neurons of the corticomedial group are generated very early in development. The differentiation of all three nuclei, however, starts some days later, probably because of the long migratory route of their neuroblasts. Evidence was provided that the neurons of the corticomedial nuclei arise from the matrix layer of the medial ventricular ridge. The neurons constituting the nuclei of the basolateral group originate later than those of the corticomedial nuclei, but their differentiation occurs at about the same time as that of the corticomedial nuclei, namely almost immediately after the arisal of the neurons. The latter are probably generated within the matrix of the lateral ventricular ridge. The nuclei of the restgroup of the amygdaloid complex differentiate in a rather late developmental period. The neurons of the nucleus tractus olfactorius lateralis as well as those of the massa intercalata are generated at the same time. The topographical relations of both grisea with the wide submaternal layer of the medial ventricular ridge suggest that the neurons constituting both grisea are derivatives from this layer. The neurons of the area amygdaloidea anterior arise at an earlier developmental period and are probably generated within the matrix layer of the medial ventricular ridge. From the fibre bundles analysed the stem bundle arises first, which is probably related to the early differentiation of certain diencephalic centres. The capsula interna, the stria terminalis and the anterior limb of the commissura anterior arise somewhat later, namely at the moment in which most of the strio-amygdaloid nuclei have started to differentiate. The posterior limb of the commissura anterior is the last to develop.

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ON THE DEVELOPMENT OF THE STRIO-AMYGDALOID COMPLEX IN THE CHINESE HAMSTER, CRICETULUS GRISEUS

PLATES

G.J. LAMMERS

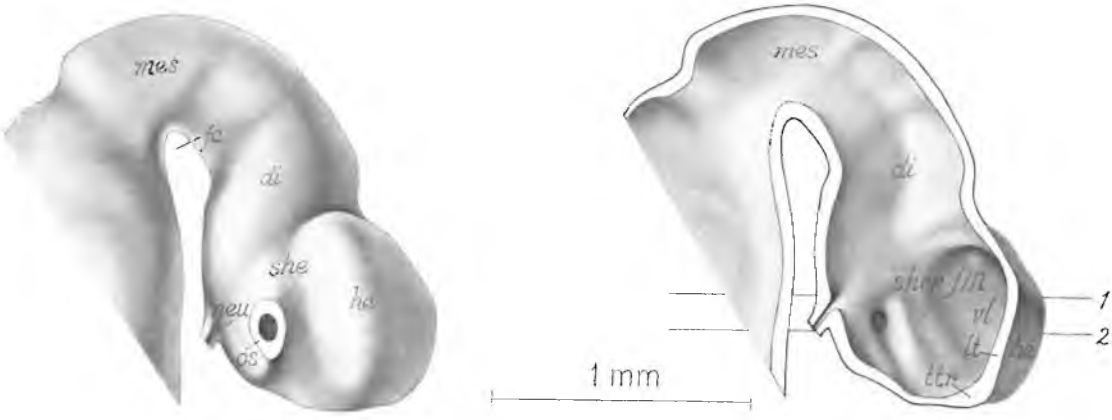
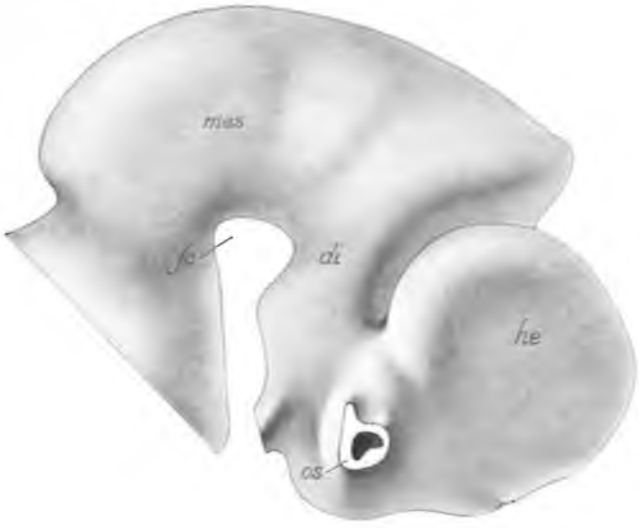


plate 1 Lateral (left) and medial view (right) of the rostral part of the neural tube at the stage E12+7h (after a three-dimensional reconstruction).



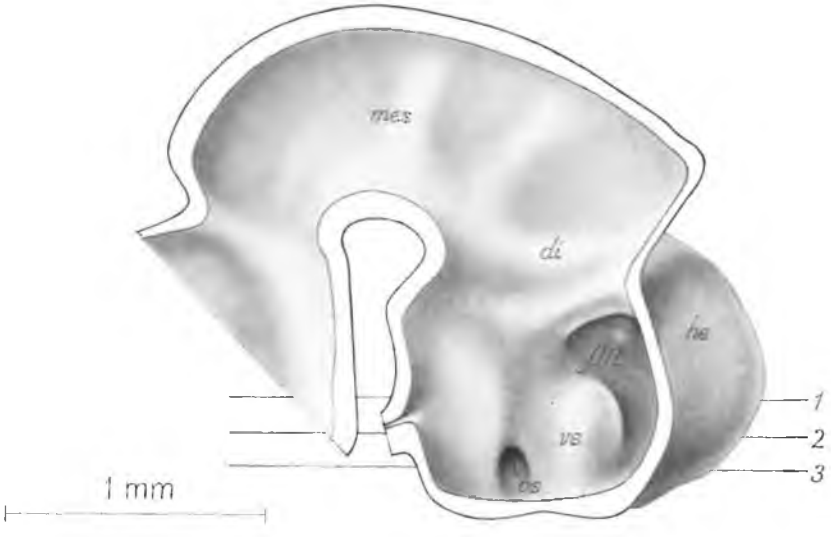


plate 2 Lateral (left) and medial view (right) of the rostal part of the neural tube at the stage E12.5 (after a three-dimensional reconstruction).

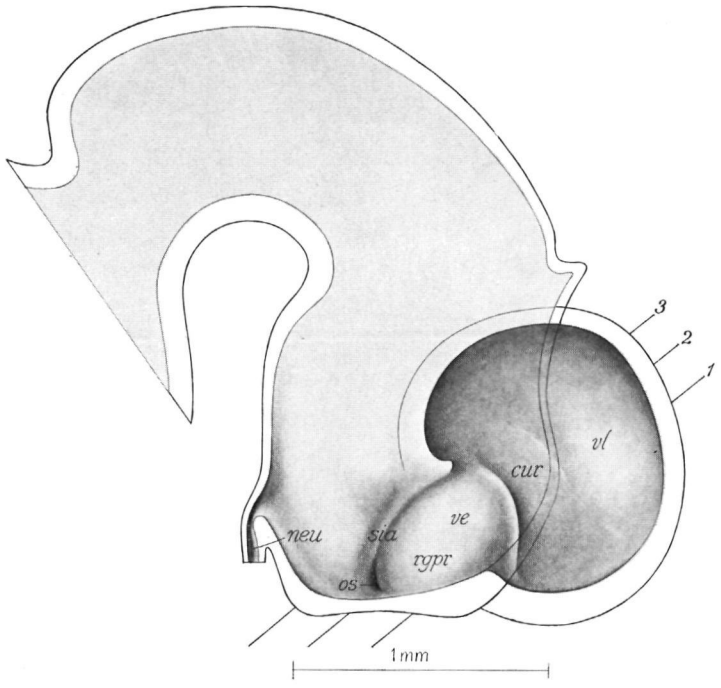


plate 3 Medial view of the rostral part of the neural tube at the stage E13 (after a three-dimensional reconstruction). The medial hemispheric wall and part of the diencephalic wall are depicted as translucent.

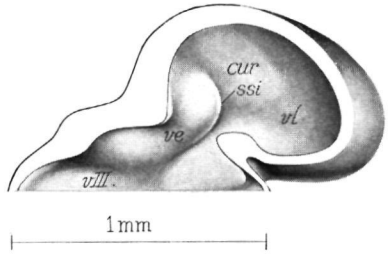


plate 4 Inner aspect of the rostral part of the neural tube at the stage E13, as viewed from above (after a three-dimensional reconstruction, cf. plate 3).

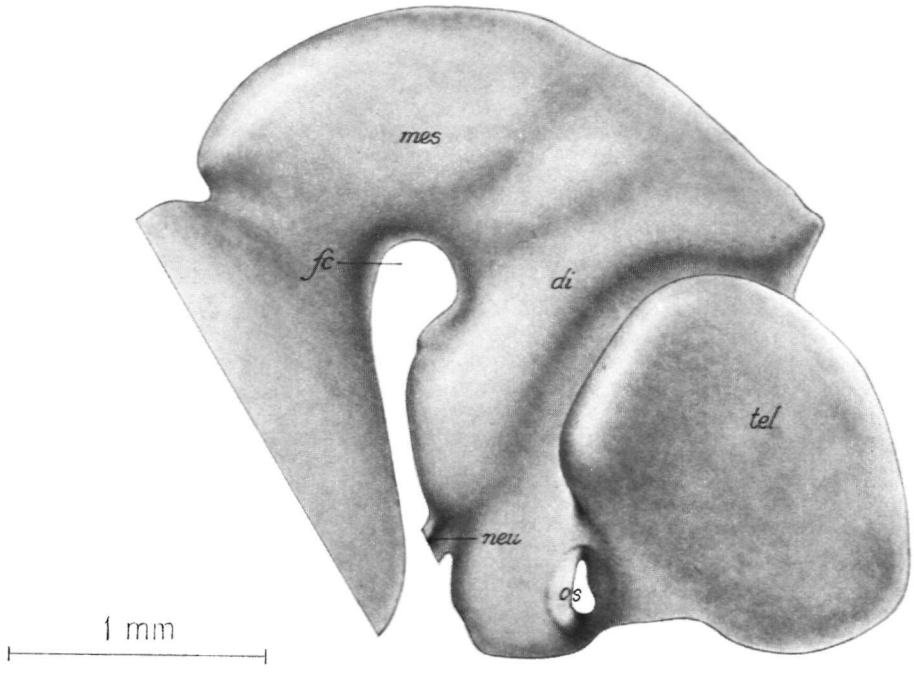


plate 5 Lateral view of the rostral part of the neural tube at the stage E13.5 (after a three-dimensional reconstruction).

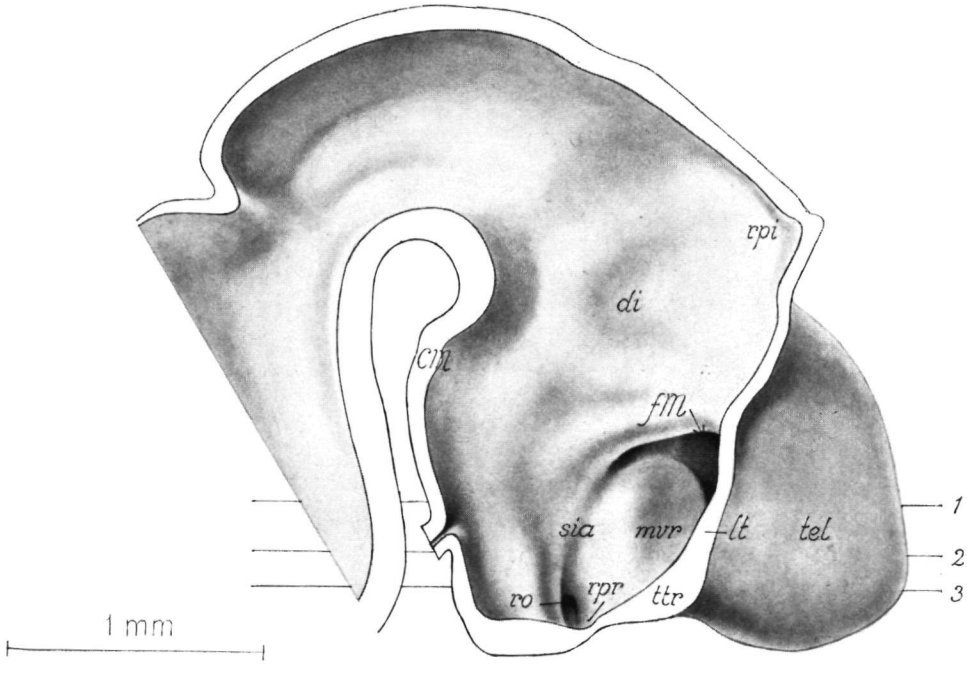


plate 6 Medial view of the rostral part of the neural tube at the stage E13.5 (after a three-dimensional reconstruction).

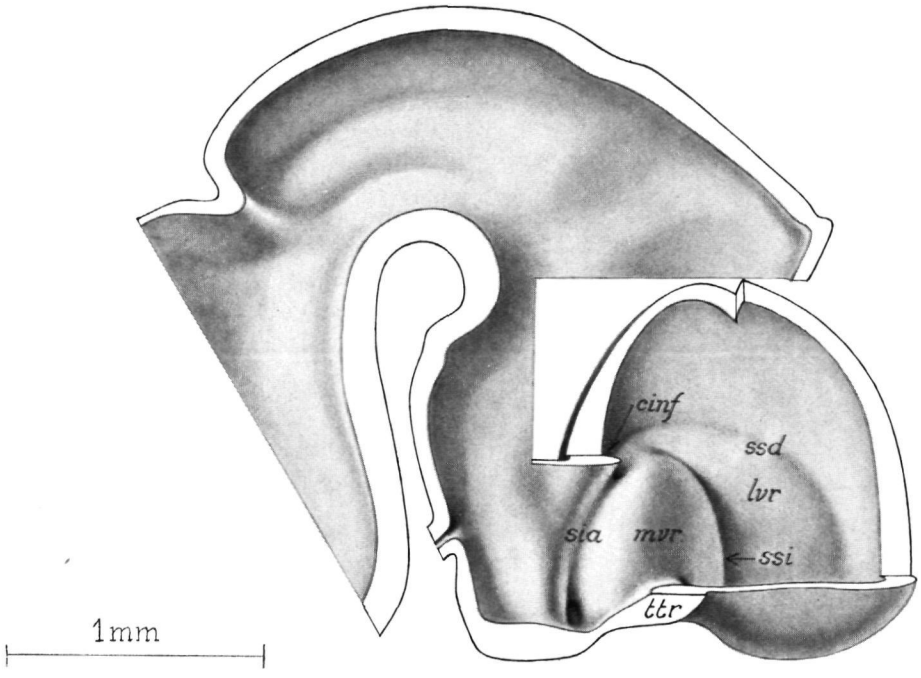


plate 7 Medial view of the rostral part of the neural tube at the stage E13.5. Parts of the medial hemispheric wall and of the diencephalic wall have been removed (after a three-dimensional reconstruction, cf. plate 6).

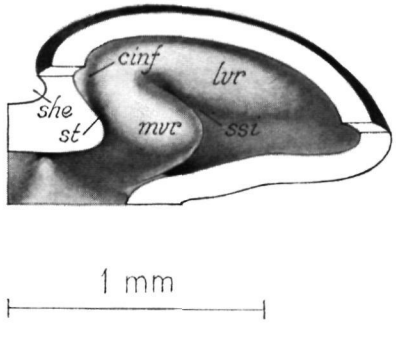


plate 8 Inner aspect of the rostral part of the neural tube at the stage E13.5, as viewed from above (after a three-dimensional reconstruction, cf. plate 7).

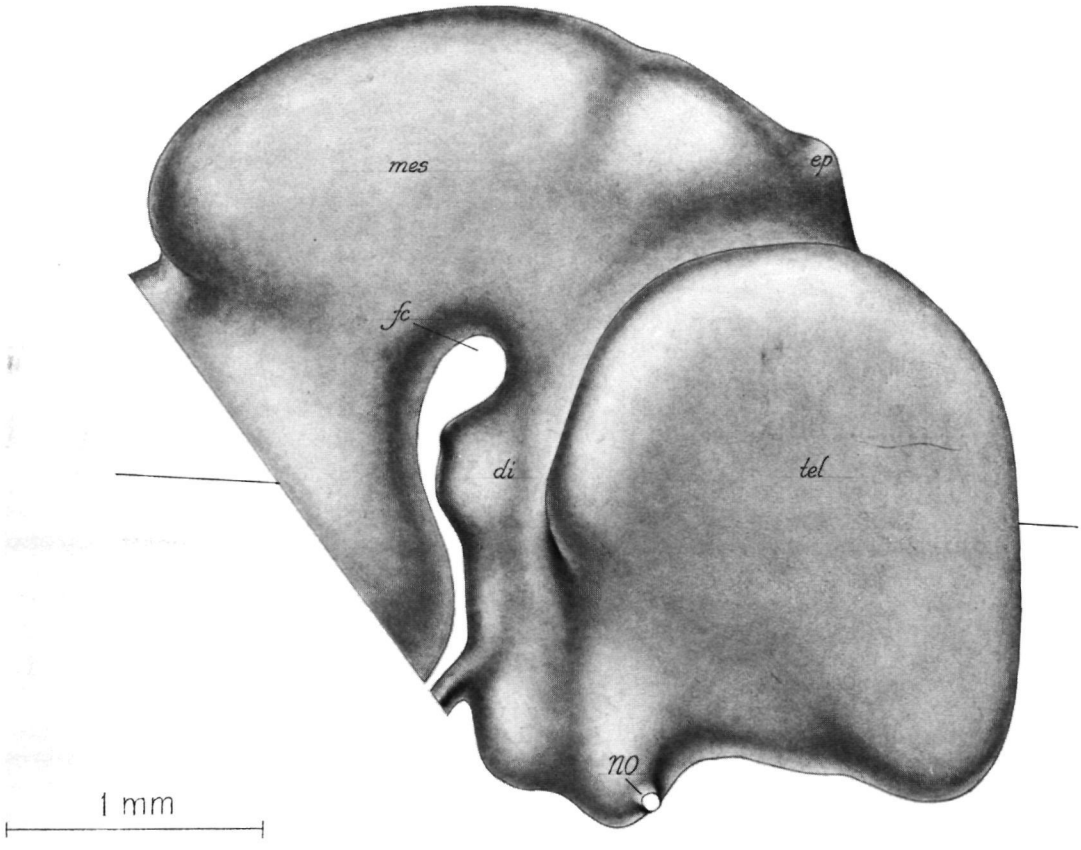
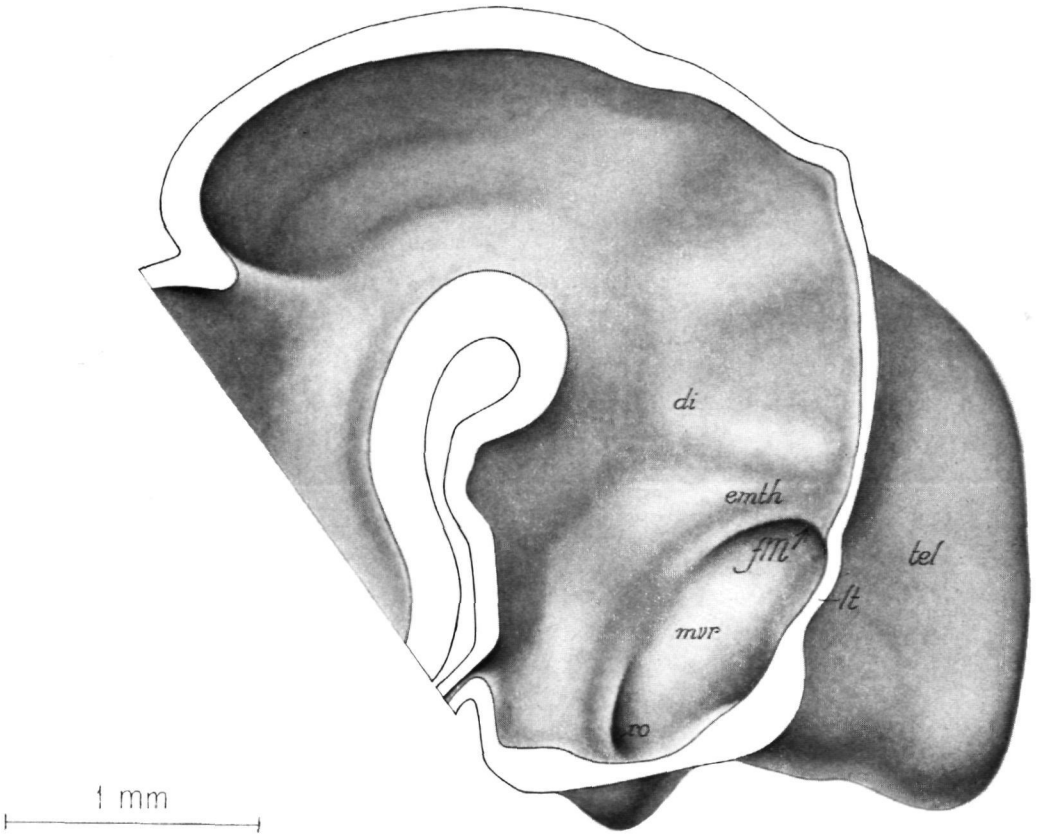


plate 9 Lateral view of the rostral part of the neural tube at the stage E14 (after a three-dimensional reconstruction).

→

plate 10 Medial view of the rostral part of the neural tube at the stage E14 (after a three-dimensional reconstruction).

plate 11 Medial view of the rostral part of the neural tube at the stage E14. Parts of the medial and lateral hemispheric wall and of the diencephalic wall have been removed (after a three-dimensional reconstruction, cf. plate 10).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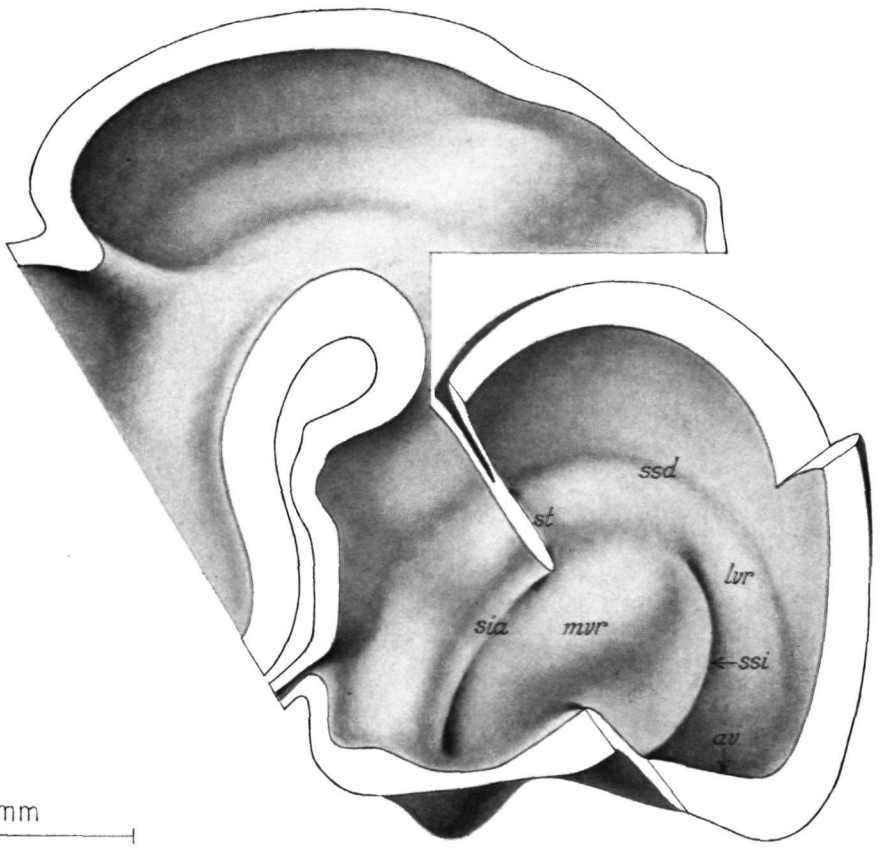
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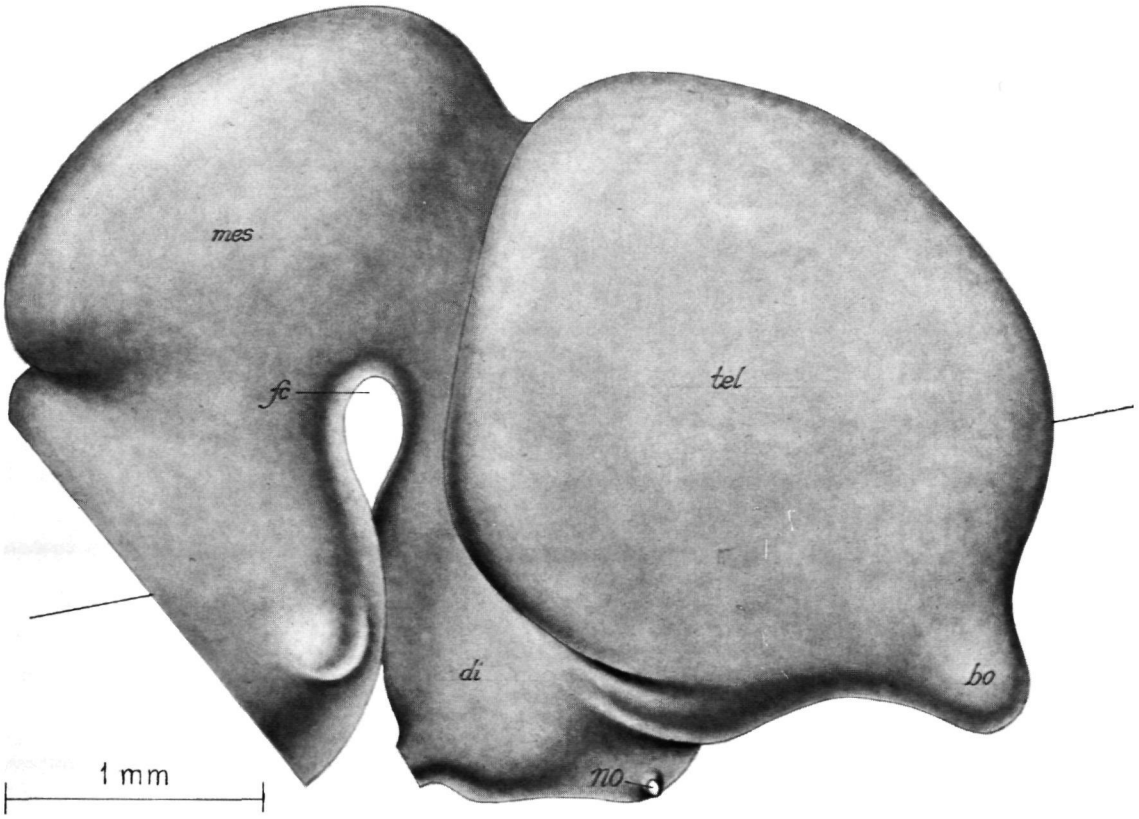


plate 12 Lateral view of the rostral part of the brain at the stage E16 (after a three-dimensional reconstruction).

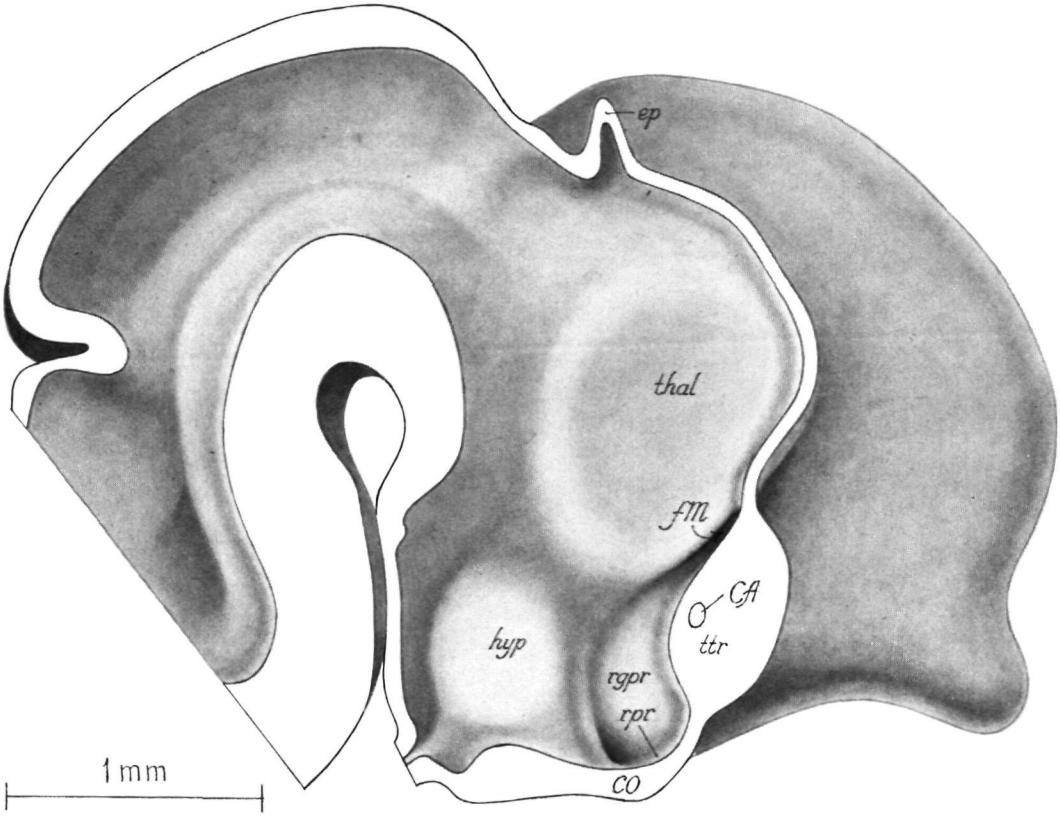


plate 13 Medial view of the rostral part of the brain at the stage E16 (after a three-dimensional reconstruction).

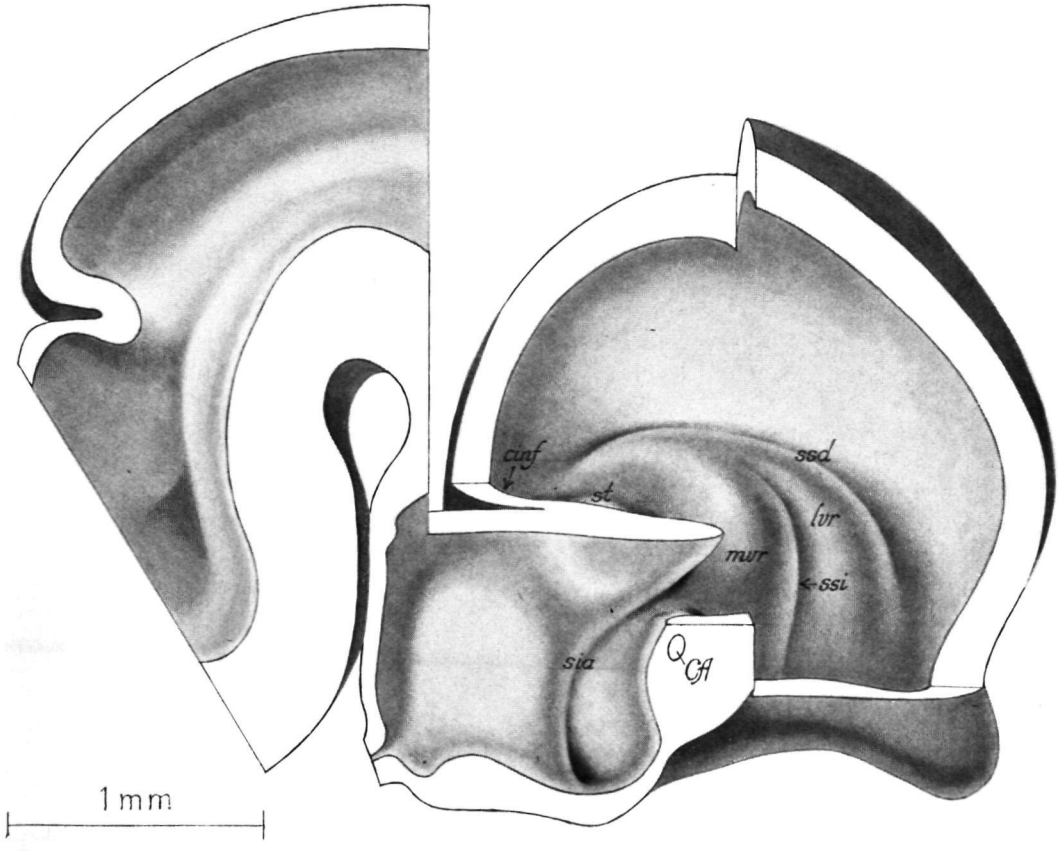


plate 14 Medial view of the rostral part of the brain at the stage E16. Parts of the medial and lateral hemispheric wall and of the diencephalic and mesencephalic wall have been removed (after a three-dimensional reconstruction, cf. plate 13).

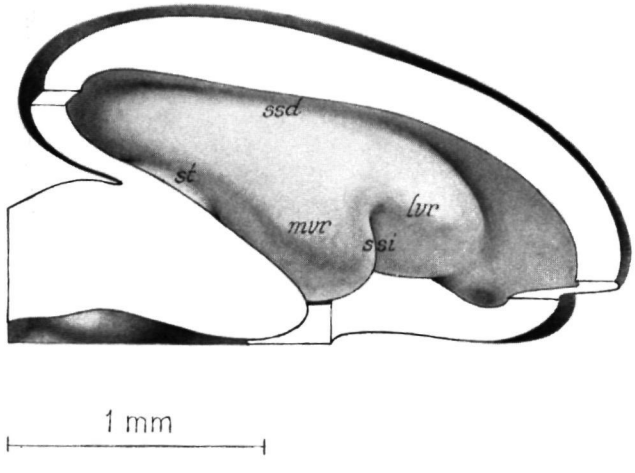


plate 15 Inner aspect of the rostral part of the brain at the stage E16, as viewed from above (after a three-dimensional reconstruction, cf. plate 14).

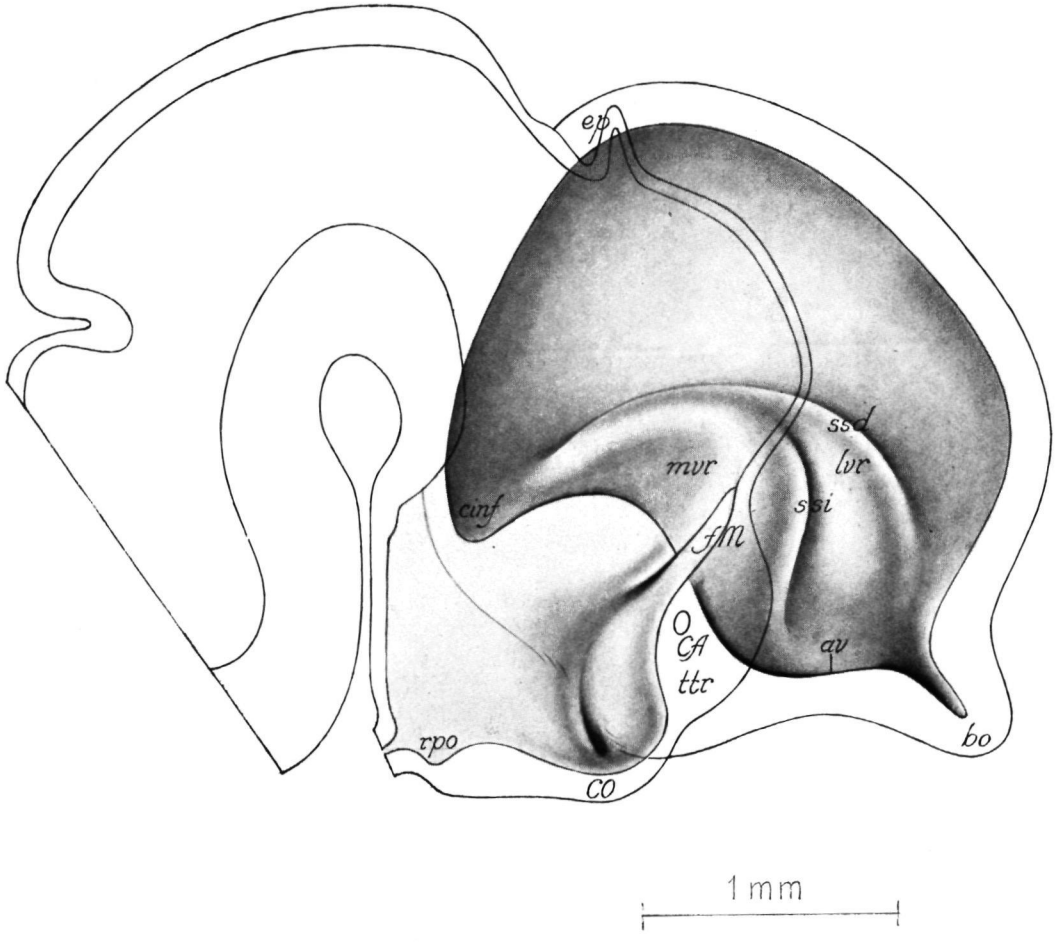


plate 16 Medial view of the rostral part of the brain at the stage E16. The medial hemispheric wall and part of the diencephalic wall are depicted as translucent (after a graphical reconstruction, cf. plates 13 and 14).

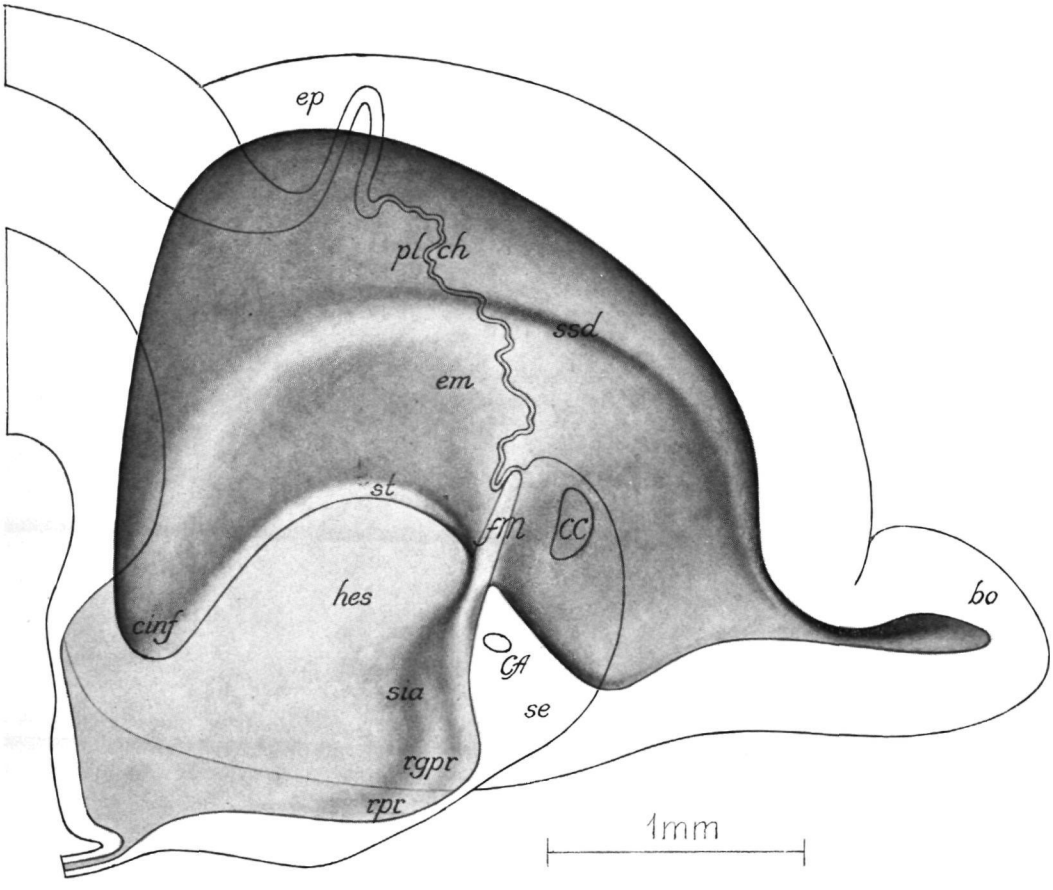


plate 17 Medial view of the rostral part of the brain at the stage E18. The medial hemispheric wall and parts of the diencephalic and mesencephalic wall are depicted as translucent (after a graphical reconstruction).

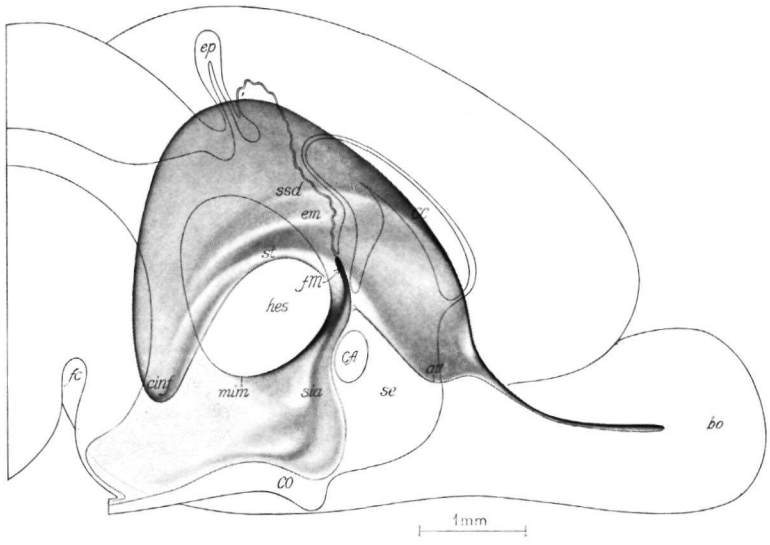


plate 18 Medial view of the rostral part of the brain at the stage PN3. The medial hemispheric wall and parts of the diencephalic and mesencephalic wall are depicted as translucent (after a graphical reconstruction).

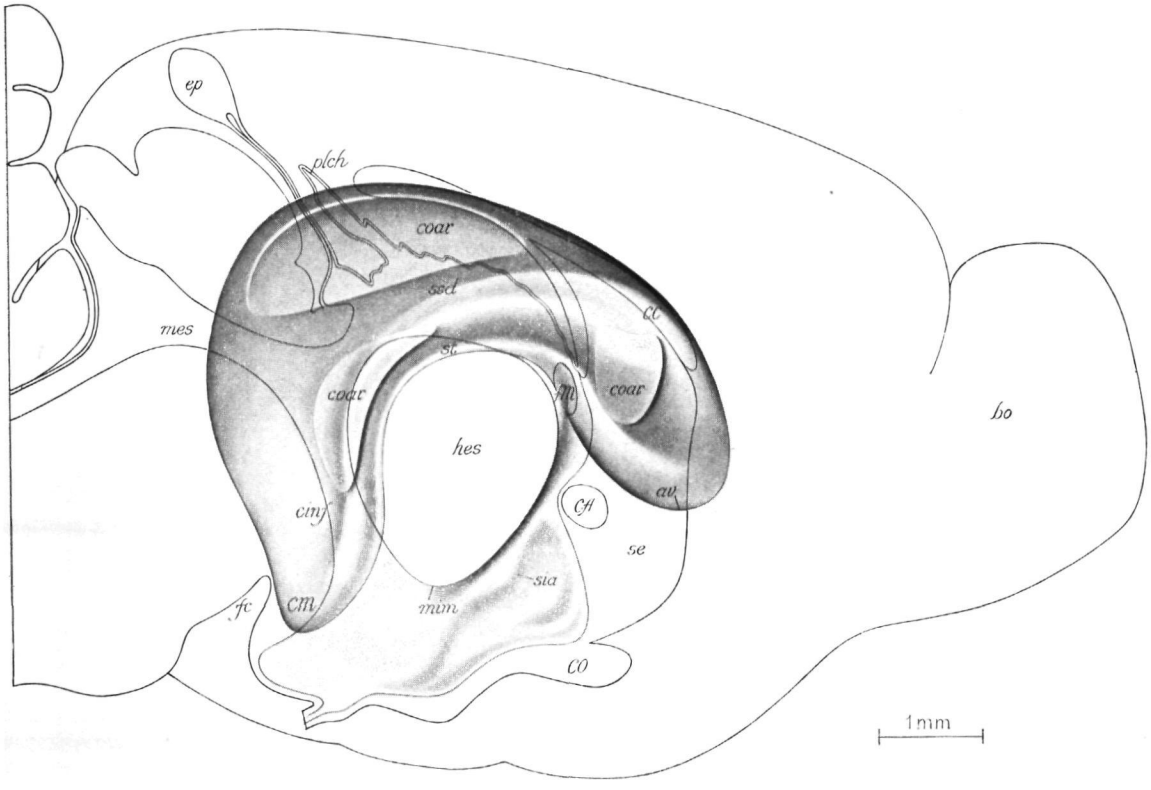
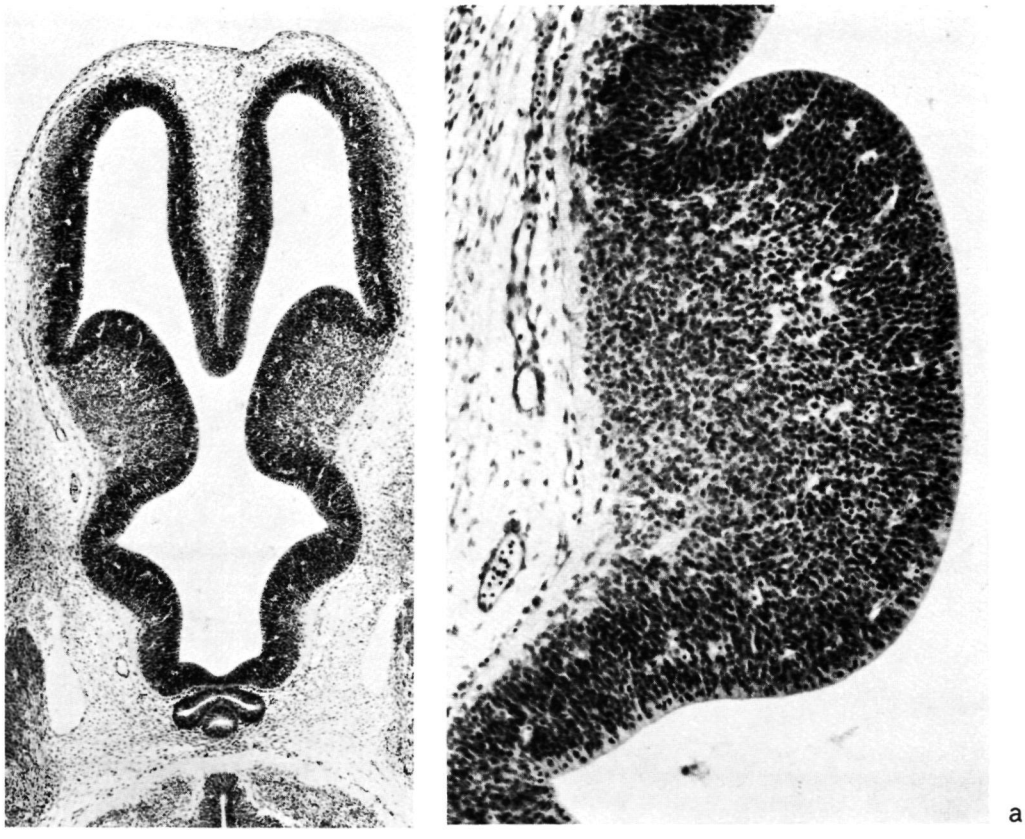


plate 19 Medial view of the rostral part of the adult brain. The medial hemispheric wall and parts of the diencephalic and mesencephalic wall are depicted as translucent (after a graphical reconstruction).



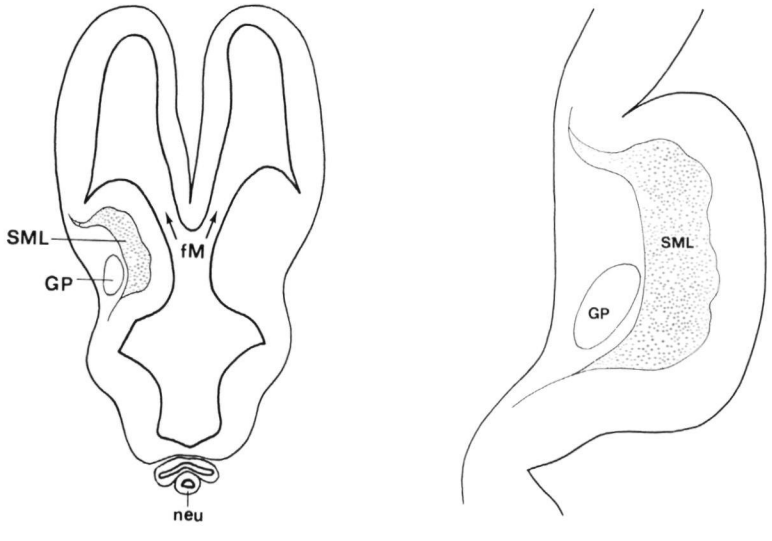
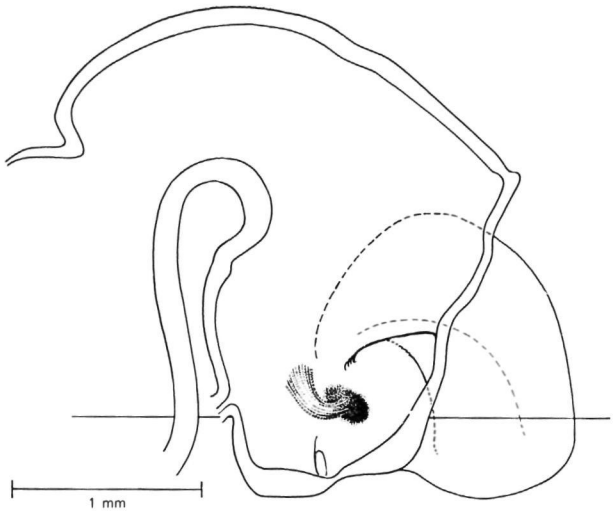
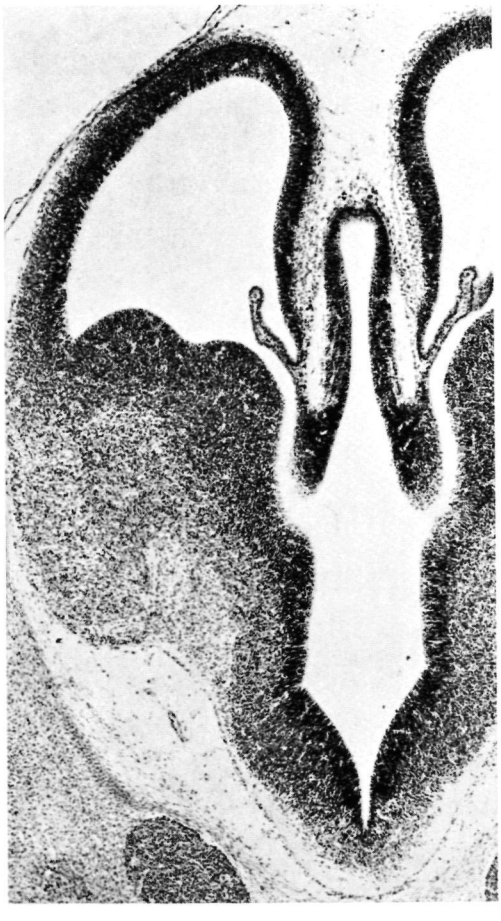


plate 20 Horizontal section through the prosencephalon at the stage E13.5 (40 x); a: medial ventricular ridge area at higher magnification (128 x). The level of the section is indicated in plate 21.

plate 21 Mantle layer structures at the stage E13.5: globus pallidus and stem bundle.





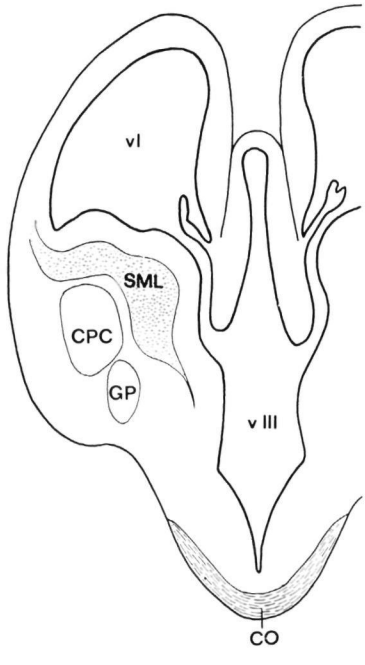


plate 22 Fronto-horizontal section through the telodiencephalic region at the stage E15 (40 x).

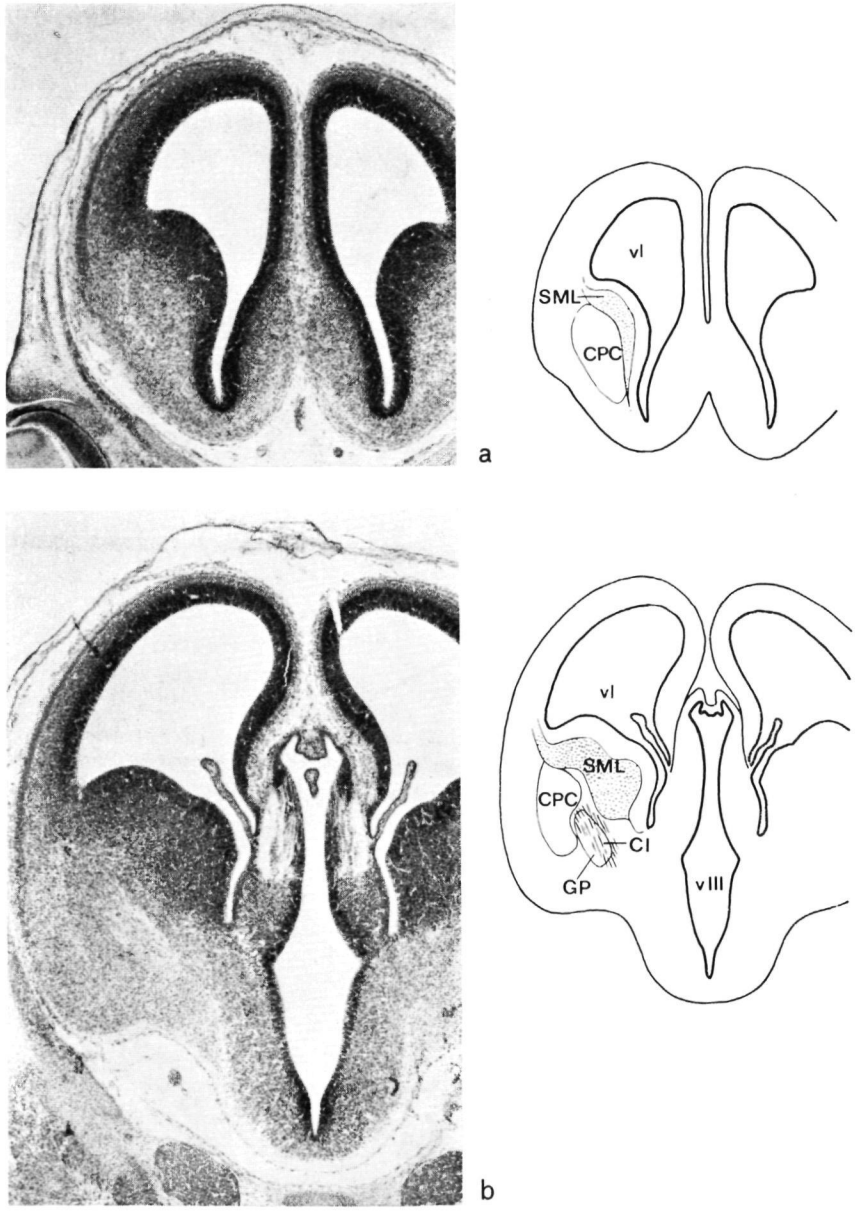
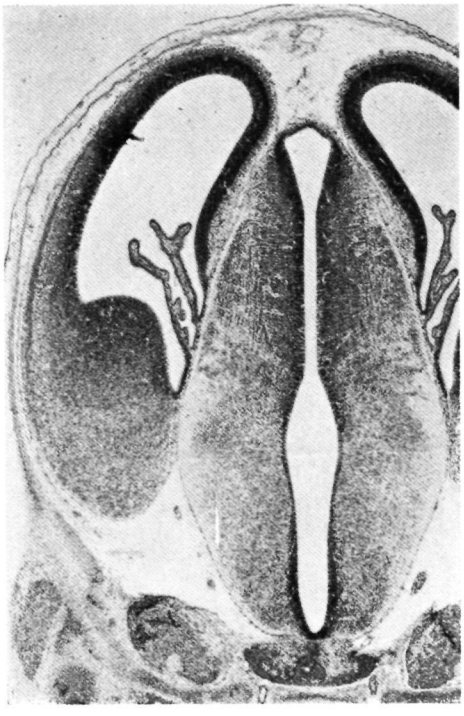


plate 23 Transverse sections through the rostral part of the brain at the stage E16 (25 x). The levels of the sections are indicated in plate 24.



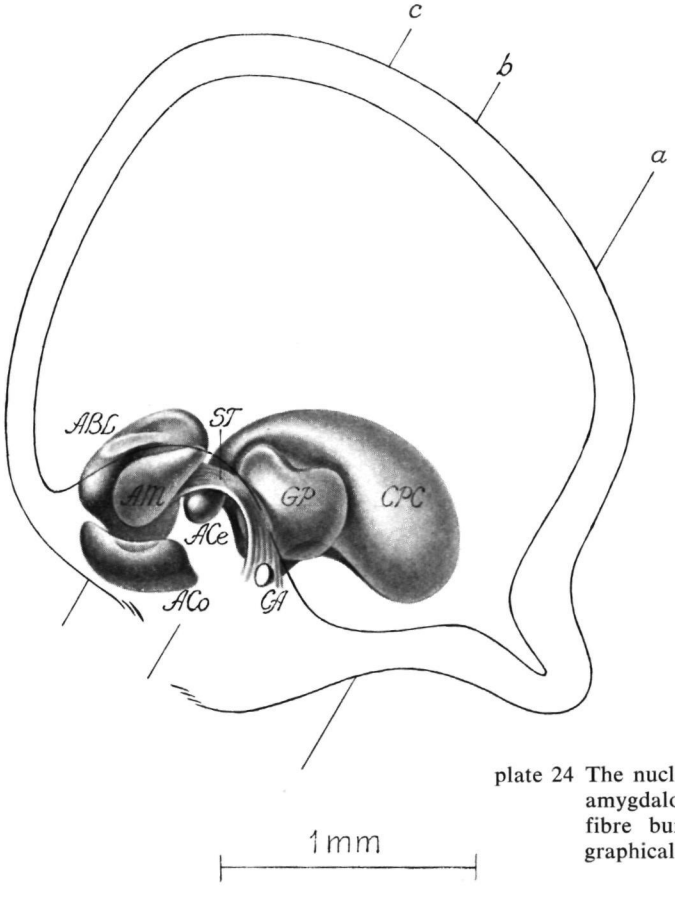
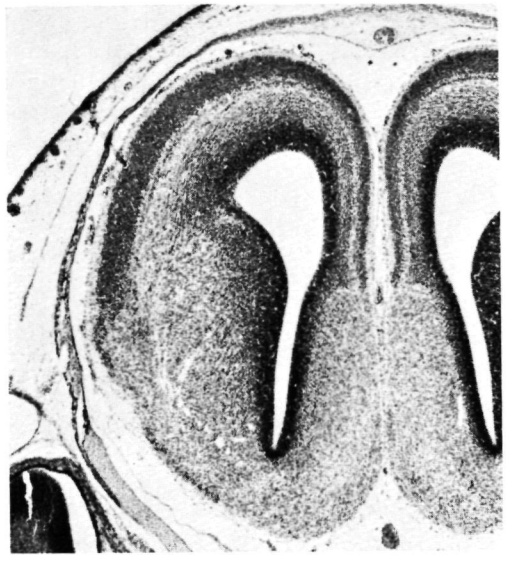


plate 24 The nuclear structures constituting the strio-amygdaloid complex at the stage E16; some fibre bundles are also indicated (after a graphical reconstruction; cf. plates 13, 14, 16).



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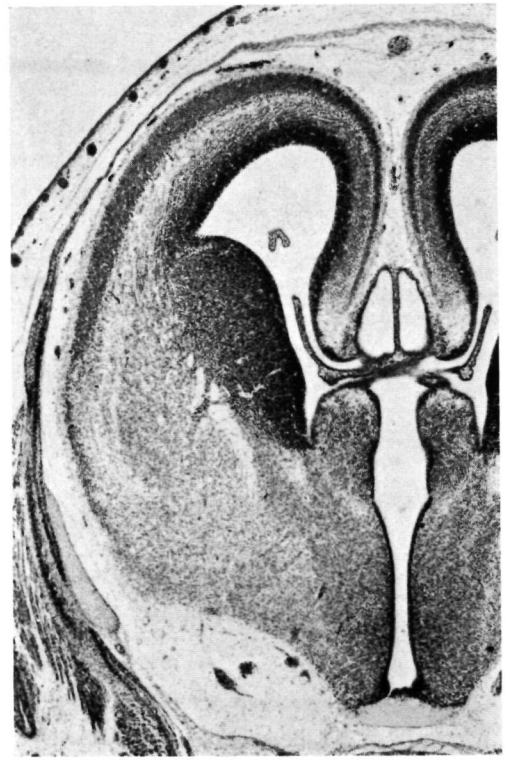

b

plate 25 Transverse sections through the rostral part of the brain at the stage E18 (25 x). The levels of the sections are indicated in plate 26.

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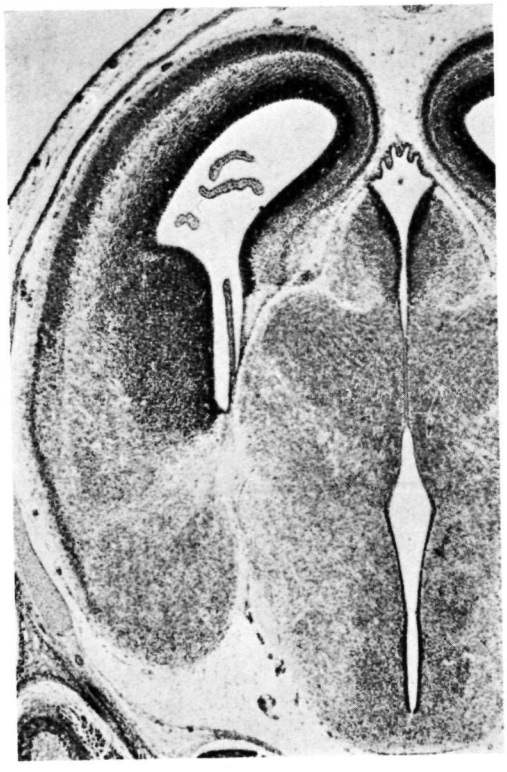
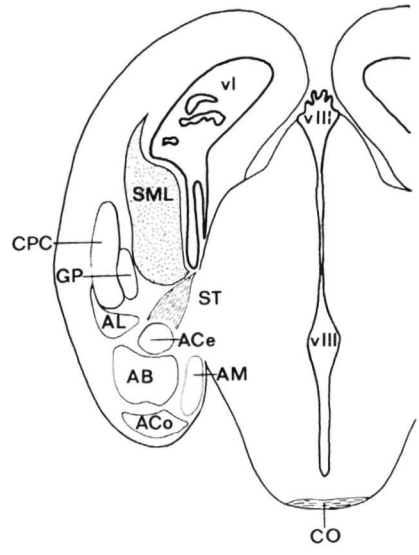
plate 26 The nuclear structures constituting the strio-amygdaloid complex at the stage E18; some fibre bundles are also indicated (after a graphical reconstruction; cf. plate 17).

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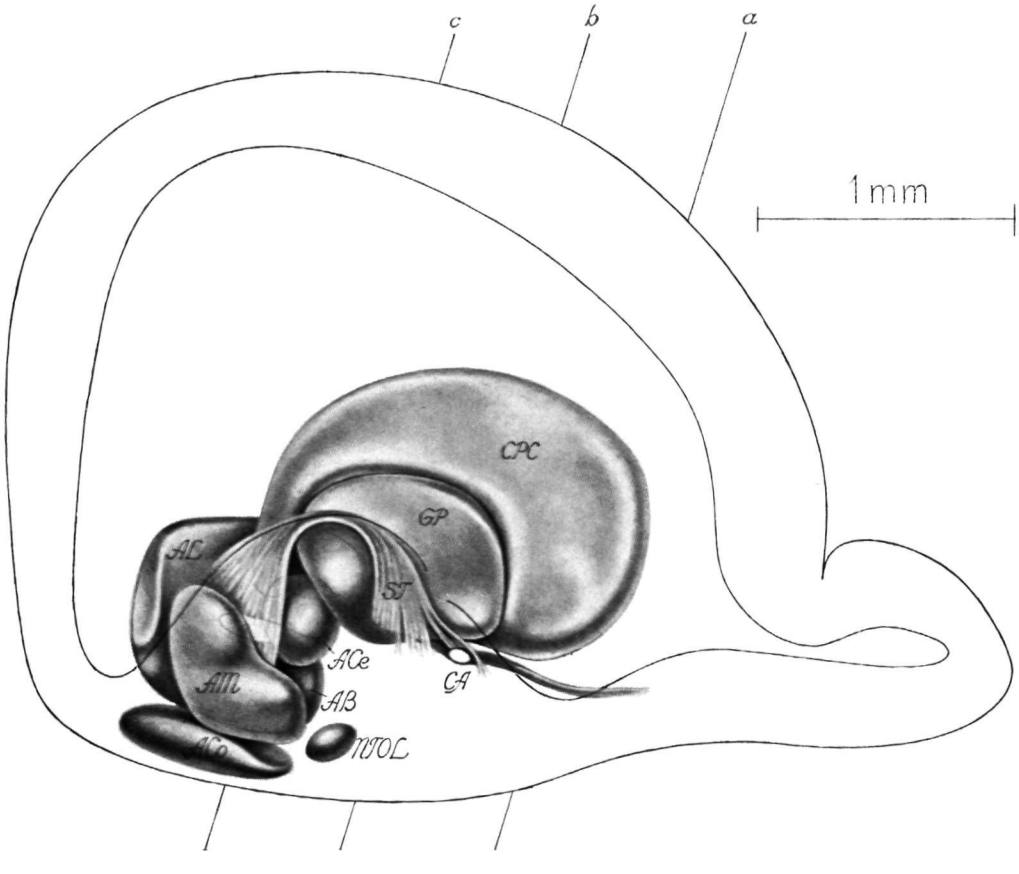
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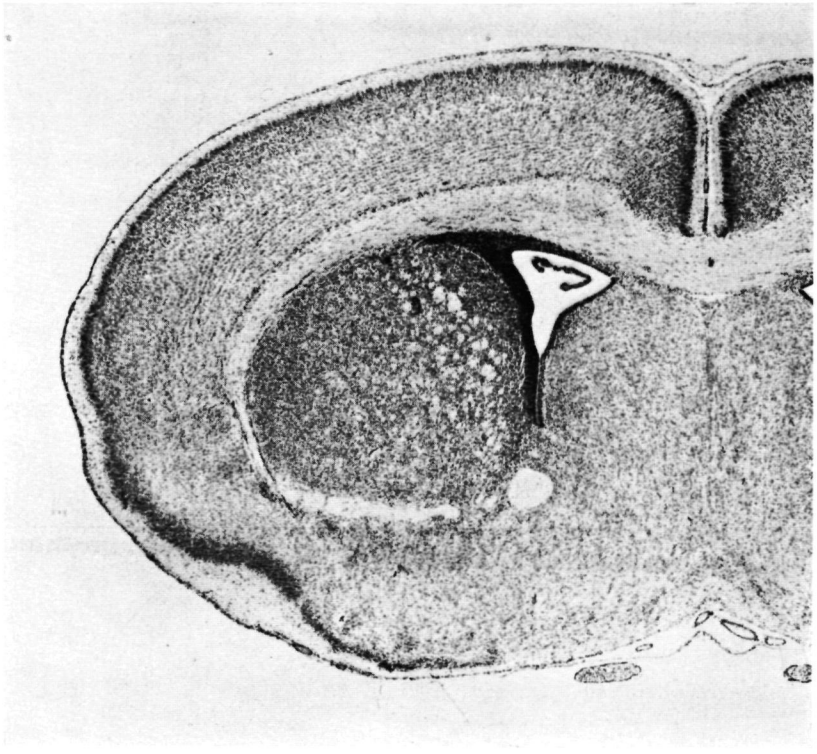

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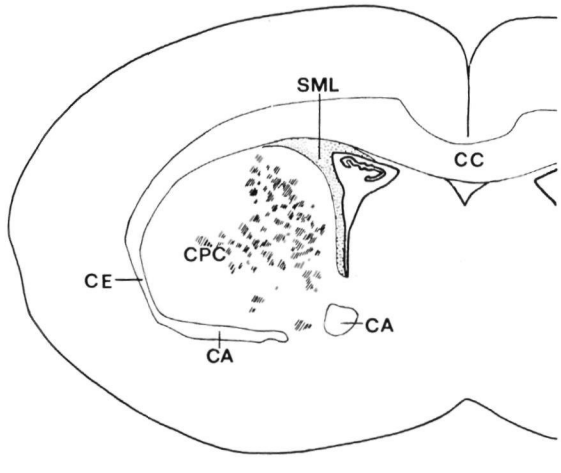
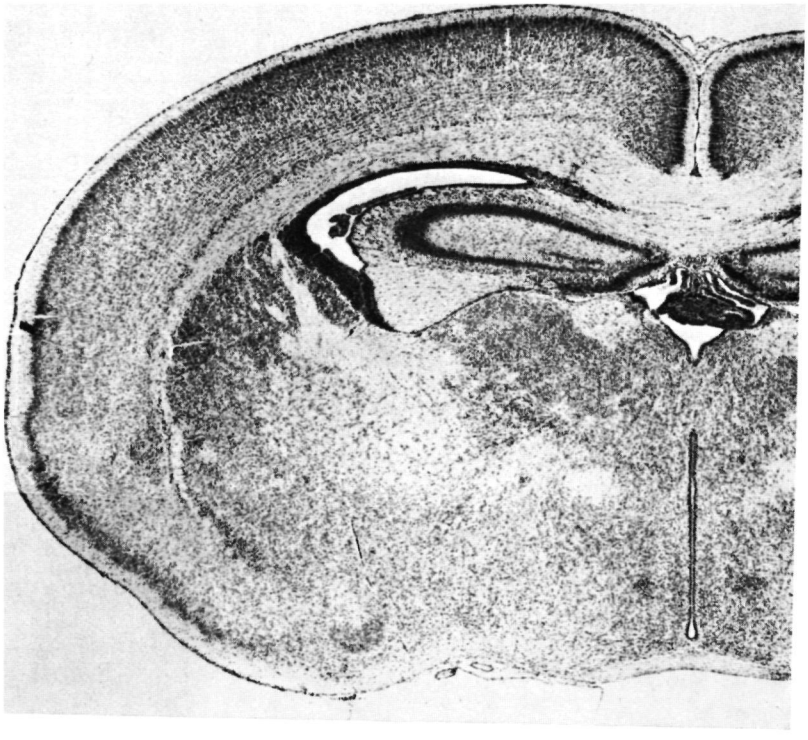


plate 27 Transverse sections through the rostral part of the brain at the stage PN3 (25 x). The levels of the sections are indicated in plate 28.



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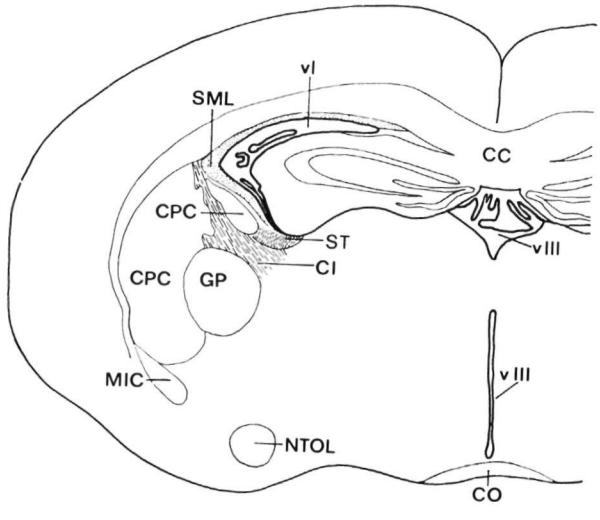

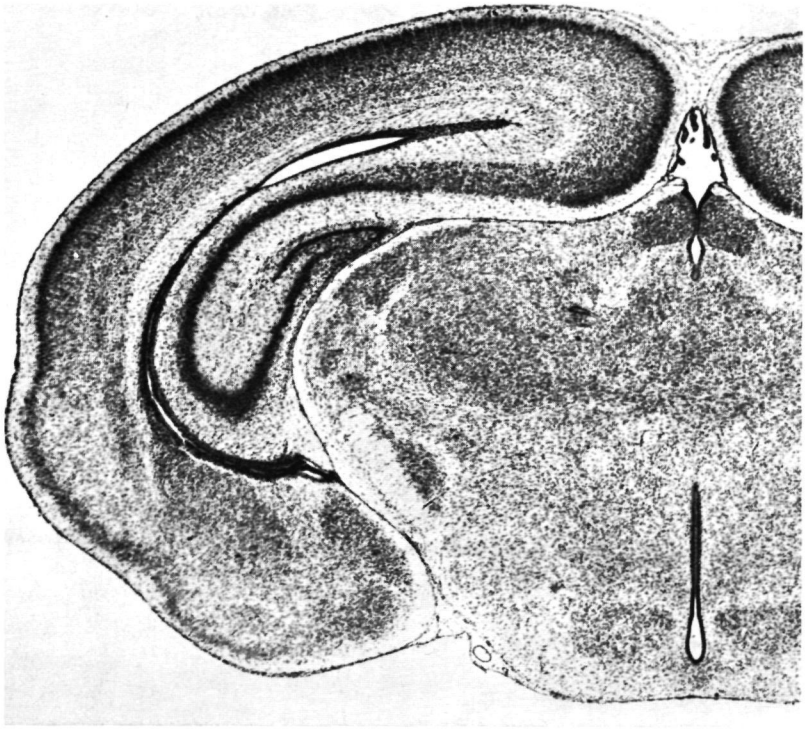

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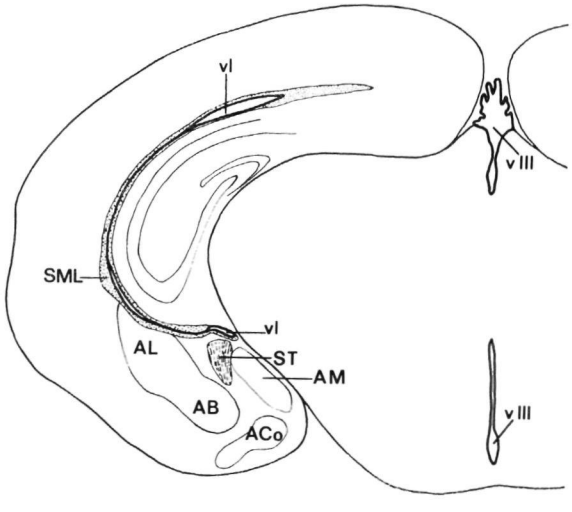


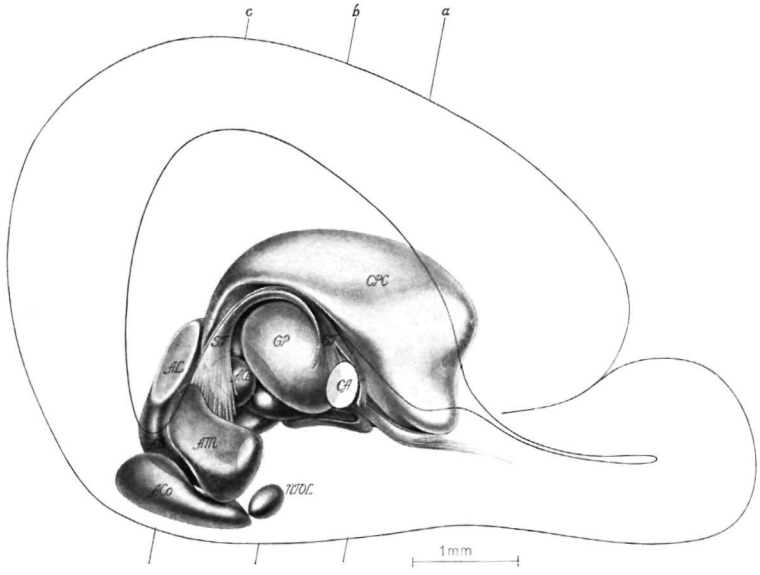
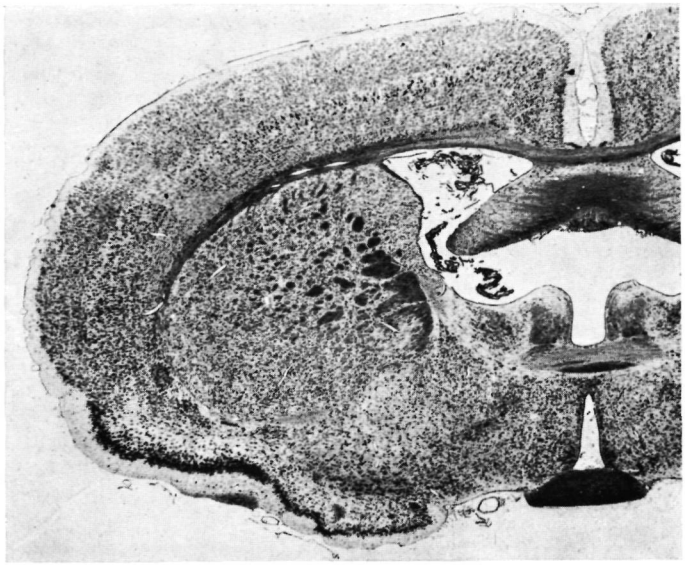


plate 28 The nuclear structures constituting the strio-amygdaloid complex at the stage PN3; some fibre bundles are also indicated (after a graphical reconstruction; cf. plate 18).



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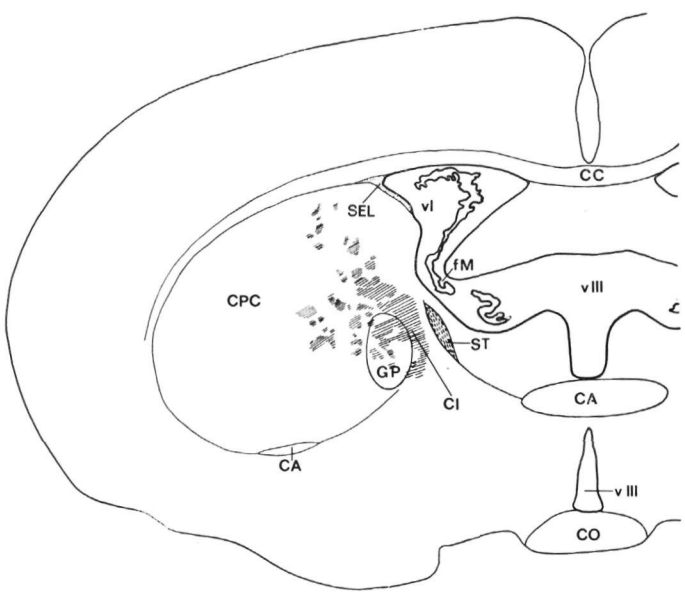
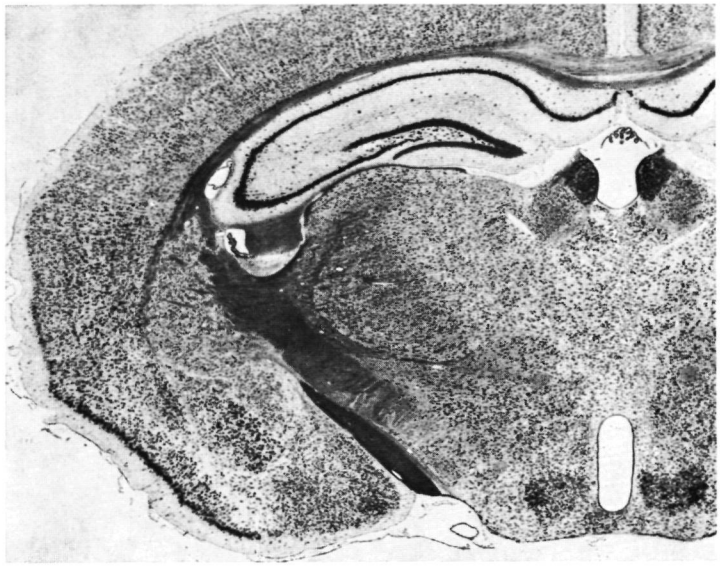


plate 29 Transverse sections through the rostral part of the adult brain (14 x). The levels of the sections are indicated in plate 30.



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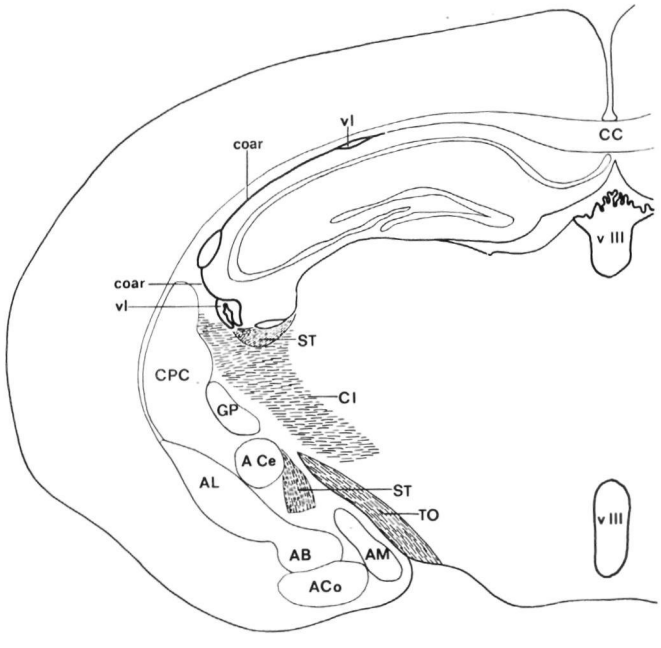

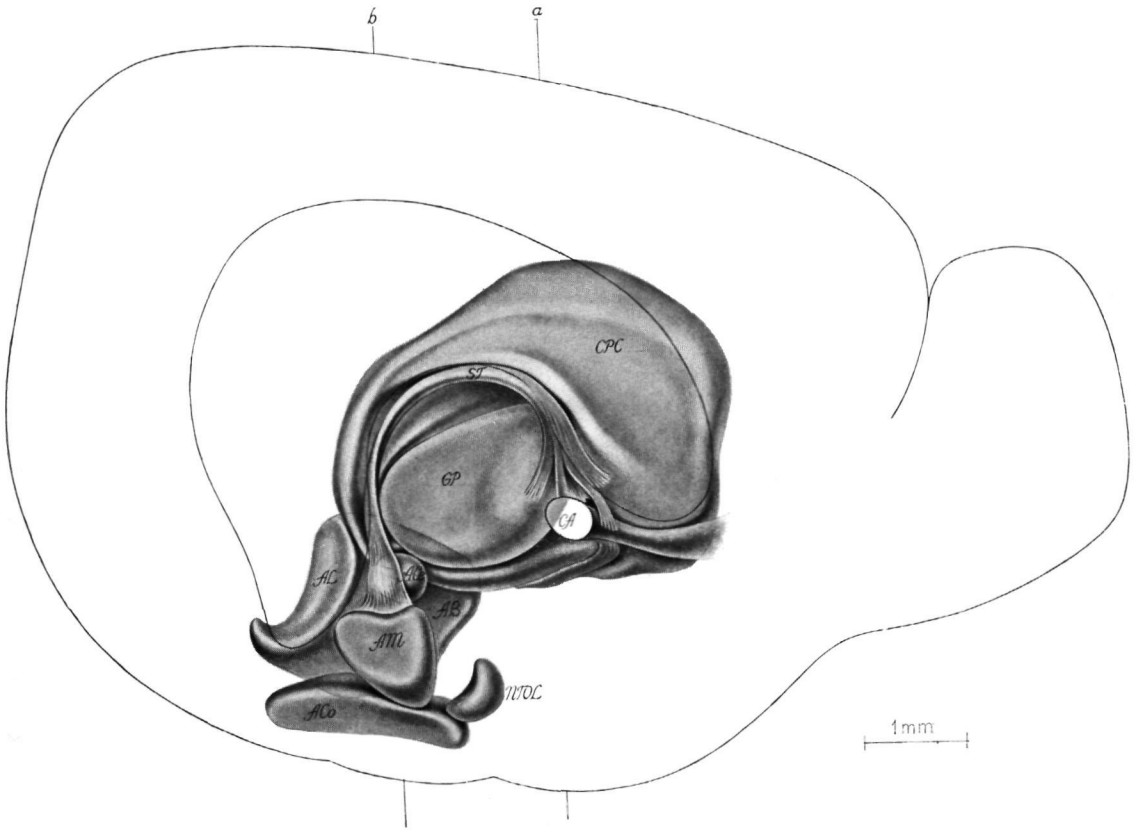

plate 30 The nuclear structures constituting the adult strio-amygdaloid complex; some fibre bundles are also indicated (after a graphical reconstruction; cf. plate 19).

STELLINGEN

I

De hypothese van Smart dat de "subependymale" laag ontstaat vanwege ruimtegebrek voor mitosen aan het ventriculaire oppervlak, wordt niet bevestigd door zijn resultaten.

Smart I (1976) J Anat 121, 71 - 84.

II

Bij genetisch bepaalde myopathiën dient uitgebreid cardiologisch onderzoek te worden verricht.

III

Medicamenteus moeilijk in te stellen vormen van epilepsie bij kinderen dienen multidisciplinair benaderd te worden.

IV

Men dient er zich rekenschap van te geven dat het angiografische onderzoek van de a. carotis een langdurig effect op de cerebrovasculaire circulatie heeft.

V

Voor een betere interpretatie van de verschijnselen voorkomend bij prikkeling van de hersenvliezen is de huidige kennis van de innervatie der leptomeningen ontoereikend.

VI

Bekendheid met gedragstherapeutische klachtenanalyse en - behandelingstechnieken is voor de praktiserend neuroloog onontbeerlijk al was het maar voor adequate verwijzing.

VII

Het aantal broedparen van de grote stern (Sterna sandvicensis) op het eiland Griend in het waddengebied (25.000 in 1939, 650 in 1965) toont duidelijk hoe gevaarlijk het is te leven als laatste schakel van de voedselketen. Het feit dat het aantal weer iets toeneemt (2.000 paren in 1973) is op zich natuurlijk verheugend, maar betekent nog niet dat het milieu-probleem in die regio nu opgelost zou zijn.

VIII

Jachtopzieners met opsporingsbevoegdheid dienen een duidelijk herkenbaar uniform te dragen bij het bestrijden van stroperij.

IX

Het populariseren van klassieke muziek wijst op een gebrek aan creativiteit.

X

De halfwaardetijd van de literatuurlijst is meestal veel langer dan die van het bijbehorende proefschrift.

Nijmegen, 18 juni 1976 G. J. Lammers